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RED ROT OF SUGARCANE

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United States Department of Agriculture, Washington, D. C.

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UNITED STATES DEPARTMENT OF AGRICULTURE

WASHINGTON, D. C.

RED ROT OF SUGARCANE [1] [2]

By E. V. ABBOTT, *associate pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry*

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INTRODUCTION

Red rot, caused by *Colletotrichum falcatum* Went, is one of the major diseases of sugarcane in the United States. Within the past decade it has caused the complete failure of one of the leading commercial varieties in Louisiana, P. O. J. 213,[3] and has shown indications of

[1] Submitted for publication August 1, 1938.

[2] The author is indebted to Robert L. Tippett, assistant scientific aide, for assistance from 1932–37 in the isolation of *Colletotrichum falcatum* Went in the survey of the red rot flora, in the preparation of culture media for the cultural studies of the fungus, and in the inoculations reported in various sections of this bulletin.

[3] Many varieties of sugarcane are commonly designated by letters or other abbreviations indicating the origin of the seedling cane. The meaning of such designations of the varieties mentioned throughout this bulletin are as follows: Co.=Coimbatore (India) seedlings; C. P.=Canal Point (Fla.) seedlings; P. O. J.=Proefstation Oost Java seedlings; P. O. J. 36–M=Mingka selection of P. O. J. 36.

increasing in severity on other varieties after a few years of large-scale commercial cultivation. Knowledge of the extent to which the failure of P. O. J. 213 and the apparently progressive increase in injury to other varieties may be due to changes in the virulence of the parasite, or the origin and increase of parasitic races specific for them, is essential for an intelligent approach to the problem of controlling the disease. Furthermore, an understanding of the nature of resistance to red rot, and the degree to which resistance is inherited in seedling progenies, is of prime importance if the hazards that the disease introduces into sugarcane culture are to be satisfactorily reduced through the breeding of resistant varieties. It is with these phases of the red rot problem that the present investigation is concerned.

DESCRIPTION OF THE DISEASE

Red rot attacks the stalks, stubble rhizomes, and leaf midribs of the sugarcane plant. It may invade leaf-blade and leaf-sheath tissues and is capable of infecting sugarcane roots (*5*, *27*, *47*),[4] but it is not important as a disease of these organs.

RED ROT IN THE STALK

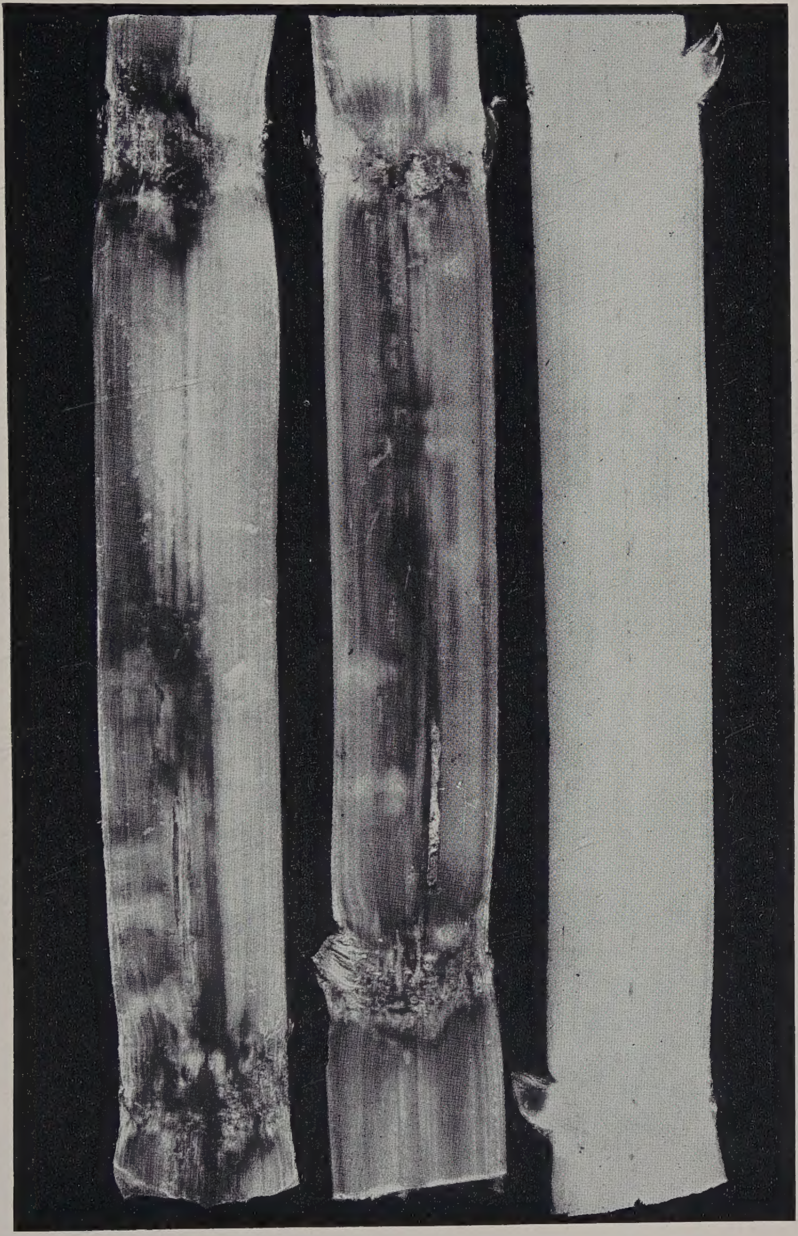
Red rot is frequently not discernible from external examination of the sugarcane stalk unless it has so completely rotted the interior as to cause the rind to lose its natural bright color and become dull in appearance. Such an advanced stage of rotting of standing cane seldom occurs in the United States, although it has been observed in P. O. J. 2714 in southern Florida. Plants so affected may be detected by the yellowing, shriveling, and dying of the upper leaves.

More certain identification of red rot may be made by splitting the stalk of standing cane, seed cutting, or stubble rhizome. The disease is recognized by the longitudinal reddening of the normally white or yellowish-white internal tissues of the internode, especially when this red color is interrupted by occasional white patches extending crosswise of the stalk (plate 1). Unless these whitish patches are present, identification as red rot is often uncertain without microscopic examination or culturing of the causative fungus. Almost any sort of wounding, including attack by other disease organisms and stalk borers, causes a red color in the adjoining tissues, which represents the natural reaction of the cane plant. However, this wound reaction is usually limited to the immediate borders of the injury; but when red rot is present, the red color may extend through many joints of the stalk, rendering it worthless for planting or milling purposes.

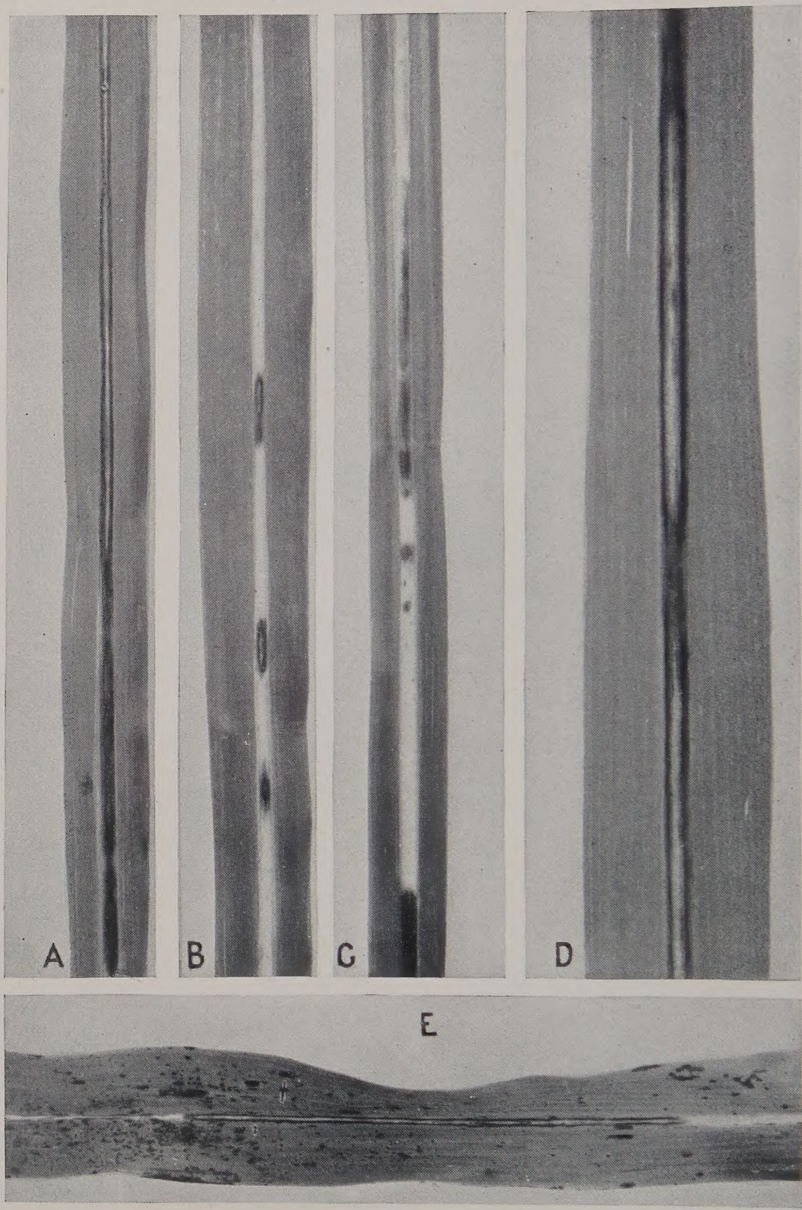
RED ROT ON THE LEAF MIDRIBS

The lesions on the leaf midribs originate as dark reddish areas, which usually elongate rapidly and sometimes extend the entire length of the leaf. The young lesions are blood red in color with darker margins. The centers become straw-colored with age, and when fructification of the fungus begins, the lesions are covered with black powdery masses of conidia (plate 2, *D*). The lesion from a single point of infection is usually continuous along the midrib, but sometimes it is broken up into a succession of red blotches alternating with

[4] Italic numbers in parentheses refer to Literature Cited, p. 93.



Typical internal stalk symptoms of the red rot of sugarcane on a very susceptible variety, showing whitish patches extending across the rotted areas and matted mycelium in the pith cavity in one of the two diseased stalks, contrasted with the natural color of a healthy stalk.



Appearance of red rot on the leaves of sugarcane and Johnson grass. The reddish areas on the upper side of the leaf midrib produce myriads of spores that are blown by the wind and constitute the principal means of spreading the disease from plant to plant. Three types of lesions are shown: *A*, Long, continuous lesion on C. P. 28/19, which is very susceptible to leaf infection but moderately resistant to red rot of the stalk; *B*, very short, restricted lesions on P. O. J. 213, which is resistant to leaf infection but very susceptible to red rot of the stalk; *C*, discontinuous lesion typical of Co. 281, which is susceptible to leaf infection but resistant to the disease in the stalk; *D*, enlarged midrib of Co. 281, showing the black, powdery masses of spores produced on the surface of the lesion; *E*, leaf of Johnson grass with typical lesion due to the same fungus.

apparently healthy tissue (plate 2, *C*). Microscopic examination of the normal-appearing areas will sometimes reveal strands of the fungus mycelium connecting the reddened spots. Frequently, however, no such connection is apparent. Atkinson and Edgerton's (*19*) demonstration of the movement of conidia in the stalks of sugarcane through the vascular bundles in the transpiration stream, lodging at intervals and initiating new points of infection, suggests that a similar movement in the leaves may be responsible for the discontinuous lesions that are commonly observed on many varieties.

In agreement with Butler's work (*27*), the author has found that isolates of the fungus obtained from leaf lesions are capable of producing red rot in the stalks, and the stalk isolates of producing the disease on the leaves. No direct mycelial connection between leaf and stalk lesions has ever been reported in the literature, and while it is not inconceivable that such might occur, it certainly is very rare. While the conidia produced on the leaves are the principal sources of inoculum for stalk infection, the leaf lesions do not ordinarily initiate stalk lesions by direct mycelial connection. The presence of the disease on the leaves cannot be taken as an indication of its presence in the stalk, nor of the susceptibility of the stalk to the disease.

LOSSES CAUSED BY THE DISEASE

Red rot causes poor stands of both plant and stubble crops as a result of the deterioration of the seed cuttings and the stubble rhizome; the destruction of seed cane in the storage beds (in the sirup-producing States); and the inversion of sucrose in mill cane, resulting in low recovery of sugar at the factory. The effect of gappy stands is felt not only in reduced tonnages of cane but also in the lower sucrose content of the juice resulting from the delayed maturity of the cane (*11*).

REDUCTIONS IN STANDS OF CANE

As early as 1909 deterioration of seed cane due to the disease was reported in both Louisiana and Georgia by Edgerton (*31*, *32*), who expressed the opinion that it had been present for some years preceding its positive identification as red rot. This opinion is borne out by the many references in trade journals to the appearance of a new rot of seed cane during the early years of the present century. Just what influence the disease had on the fluctuating yield curve in Louisiana, prior to the time of the complete failure of the noble canes from a combination of diseases in 1926, cannot be accurately determined, but reports of epidemics of red rot during these years indicate that it was an important contributing factor to their decline. According to Edgerton, Taggart, and Tims (*37*), the season of 1923 witnessed one of the worst red rot epidemics in the history of Louisiana, while Tims and Edgerton (*67*) stated that stands of cane in 1924 were reduced nearly 50 percent by the disease.

During the period 1927–29 less serious losses were experienced by the industry because of the replacement of the noble canes with the P. O. J. varieties. Injury to the old varieties was severe in 1927 (*2*, *40*), but because of the rapidly increasing acreages of the newer canes, which gave good stands, the effect was less felt by the then reviving industry. According to Tims and Edgerton (*67*), however, a considerable portion of the Louisiana Purple produced in 1927 was

so severely rotted that it was not worth sending to the mill. Conditions continued to improve until the spring of 1930, when thousands of acres of P. O. J. 213 that had become one of the leading commercial varieties of Louisiana either completely failed or were so reduced in stand as to make their cultivation unprofitable (fig. 1). The tremendous cost of this failure to the industry may be appreciated by an examination of figure 2, in which the percentage of the total acreages of sugarcane in Louisiana occupied by P. O. J. 213 and certain other varieties from 1928–36 are graphically recorded.

It will be noted that by 1929, P. O. J. 213 occupied a third of the entire sugarcane acreage of the State, and in 1930, the year of its first disastrous failure, 35 percent. Probably no one has ever attempted to estimate the exact economic loss involved in this failure, since figures are not available from a sufficiently large number of plantations with respect to the number of acres actually plowed out or the acreage which, in spite of the greatly reduced stands, was cultivated at a cost considerably above normal. Nevertheless, some idea of the monetary loss may be gained when it is considered that it costs approximately $11 to $12 to plant an acre of cane, including the cost of land preparation, the cane used for seed, cutting, hauling, and planting, and an additional $30 per acre for producing the preceding legume crop, for drainage, and the various fixed administrative costs, which must be charged against the plant-cane crop. On this basis, the cost of producing the 1930 crop of P. O. J. 213 of approximately 25,500 acres may be estimated at approximately $1,058,250.[5] On some plantations this cost was nearly a total loss, and on all but a very few it was considerable.[6] The loss to the sugar district as a whole from the failure of P. O. J. 213 in 1930 and 1931 may be conservatively estimated at from $635,000 to $1,100,000. It was a severe blow to an industry not yet fully recovered from the debacle of 1926.

This initial loss, however, was not all, for the effects of a poor stand of plant cane are carried into the succeeding stubble crops. In order to maintain the normal balance between plant cane and stubble crops, it was necessary for many plantations to carry stubbles of P. O. J. 213 in which the stands were poor. This meant that the costs of cultivating, fertilizing, hoeing, and other field operations were greatly increased, even more perhaps than in the plant cane crop.

The 1930 experience with P. O. J. 213 was repeated to a considerable degree in 1931, when 41 percent of the total sugarcane acreage in Louisiana was still devoted to this variety, and to some extent in succeeding years. The natural result was a sharp decline in acreage of P. O. J. 213, until by 1934, when it was again severely affected by red rot, it had ceased to be an important commercial variety. Aside from the economic aspects of the failure of this variety, its loss was extremely discouraging to an industry, in the recovery of which it

[5] According to the United States Department of Agriculture Yearbook for 1934, there were 150,000 acres devoted to sugarcane in Louisiana in 1930. Assuming that the crop was 40 percent plant cane and 60 percent stubble, this would mean approximately 60,000 acres of plant cane. On the 62 plantations, for which figures are available in Gilmore's Manual of Louisiana for 1930–31, P. O. J. 213 constituted 42.6 percent of the plant cane crop. Applying these figures to the total State acreage, there were approximately 25,500 acres of P. O. J. 213 plant cane. At an average cost of $41.50 per acre, the cost of producing the 1930 crop of this variety was approximately $1,058,250. Similarly, the cost of producing the 1931 crop of P. O. J. 213 of 18,200 acres was approximately $755,300.

[6] In correspondence, several plantation managers have estimated their losses from red rot injury to P. O. J. 213 in 1930 as from 35 to 60 percent.

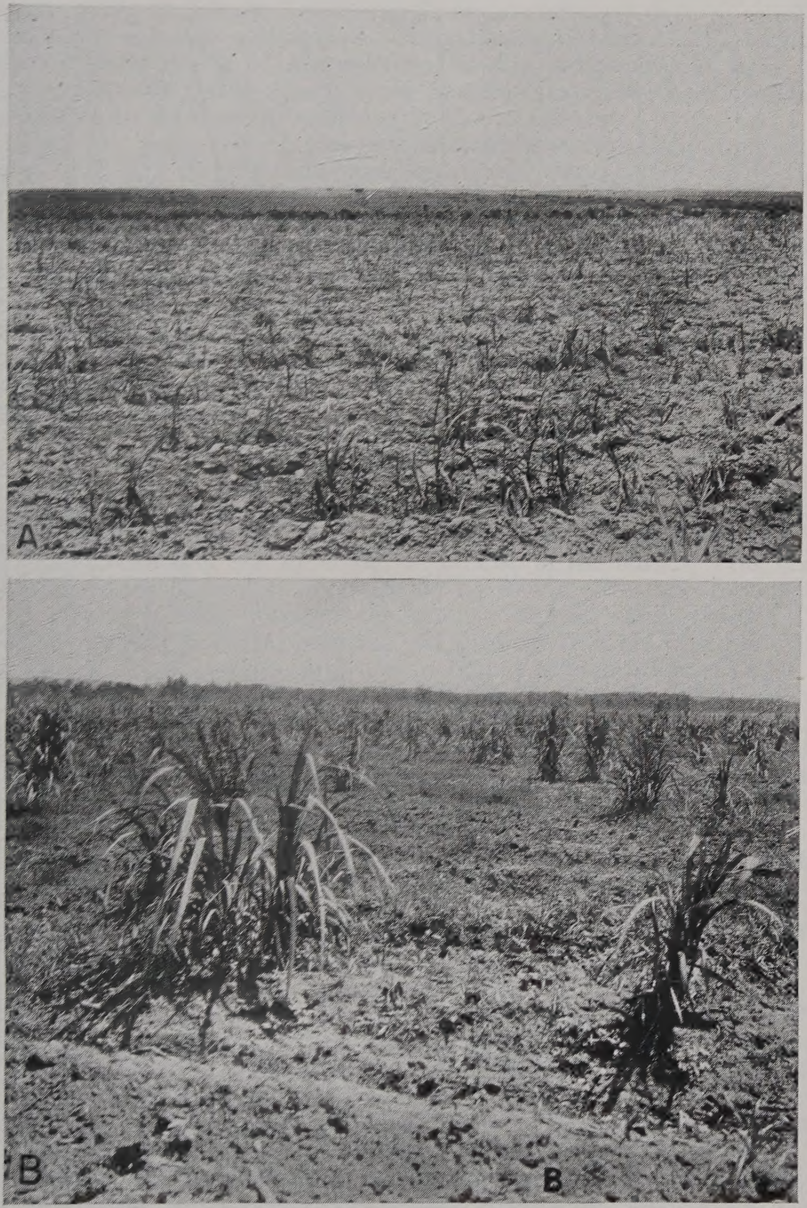


FIGURE 1.—Disastrous effects of red rot on stands of P. O. J. 213 in southern Louisiana: *A*, Plant cane in late April (looking across the rows) showing gaps of from 4 to 20 feet in many of the rows; *B*, a view of a field of second stubble, showing loss of stand due to stubble deterioration in addition to the original decimation of the plant-cane stand.

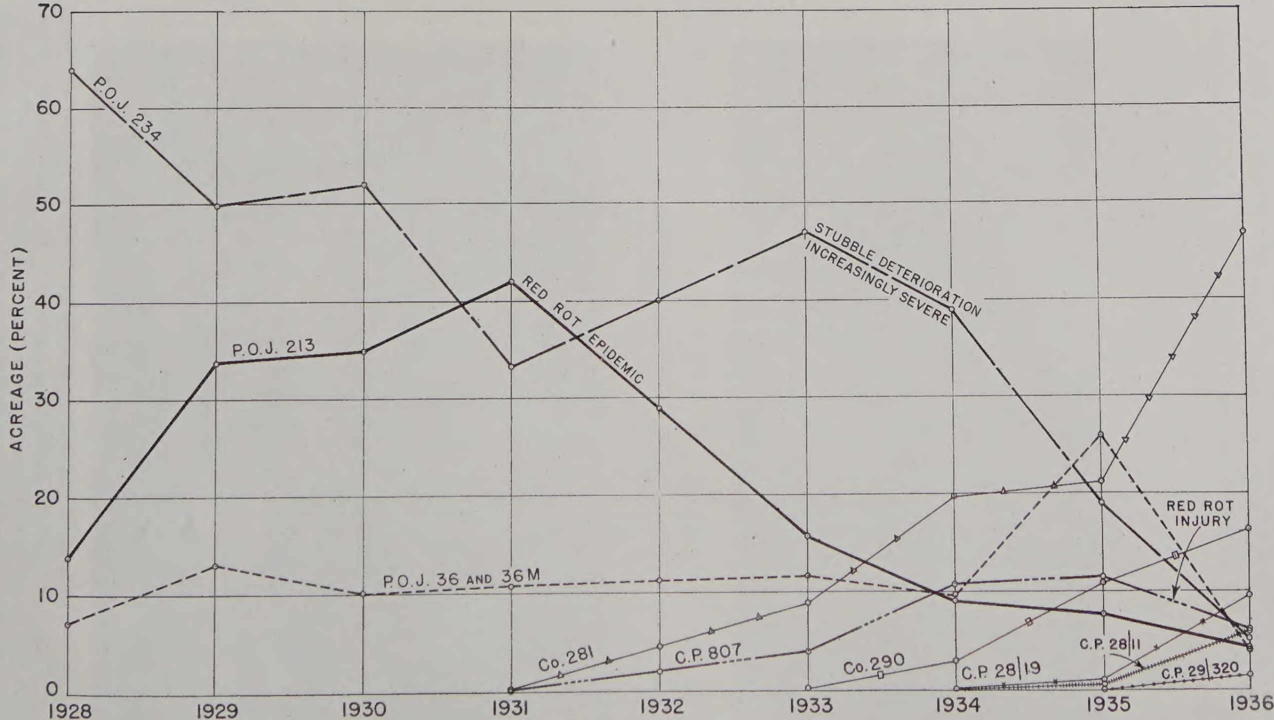


FIGURE 2.—Percentage of total sugarcane acreage in Louisiana occupied by the commercial varieties, 1928–36, showing the varietal succession necessitated largely by failure due to diseases. The outstanding varietal failures were of P. O. J. 213 and P. O. J. 234, the former being due primarily to red rot, and the latter to stubble deterioration. The dip in the curve for P. O. J. 234 in 1931 was due more to the increasing popularity of P. O. J. 213 than to disease factors of the former, and the corresponding rise in 1932–33 resulted from the disastrous failure of P. O. J. 213. The increasing failure of P. O. J. 234 necessitated greater utilization of the less desirable P. O. J. 36 and P. O. J. 36–M in 1935 until the later introduced Co. 281 and Co. 290 could be multiplied. (Data mainly from Gilmore's Sugar Manual of Louisiana.)

had played so important a role, and naturally led to apprehension on the part of growers that the P. O. J. varieties would follow the noble canes in failure. Subsequent events showed that this feeling was not entirely unjustified. Fortunately, however, the United States Department of Agriculture breeding program had then progressed to the point where more resistant canes were immediately available to replace the declining P. O. J. varieties.

Red rot losses during the past decade have not been confined to P. O. J. 213. During the epidemics of 1929–30 and 1930–31, P. O. J. 36–M, which is slightly more resistant than P. O. J. 213, also suffered from the disease, but because of the relatively smaller acreage devoted to the variety, the effect on the industry was not so marked. Again in 1934 several hundred acres of plant cane of P. O. J. 36–M showed reductions in stands estimated at from 30 to 70 percent.[7] In 1935 severe red rot injury to stands of both plant cane and stubble crops of C. P. 807 resulted in a sharp decline in acreage of this variety in subsequent years, complicating the problem of finding suitable varieties for heavy soils.

LOSSES IN SUCROSE

In addition to the loss in tonnage due to reduced stands of cane, red rot also causes important reductions in recoverable sugar at the factory because of the inversion of sucrose in the stalk. This phase of the subject was first investigated by Went (*73*), who found that the disease greatly lowered the quality of mill cane. Butler (*27*) confirmed this in India, pointing out that the damage was due to the inversion of the sucrose and not to actual consumption of sugar by the fungus. Lewton-Brain (*46*), in Hawaii, and Edgerton (*33*), in Louisiana, reported similar results. Edgerton found not only that the sucrose content of the diseased internodes was reduced, but that the joints above the diseased ones were also affected. In the epidemic year of 1927 in Louisiana, the reductions in sucrose in juices of the noble varieties were as high as 33 percent (*2*).

McKaig and Fort (*48*), working with varieties of varying susceptibility to red rot (P. O. J. 213, 36–M, and Co. 281), found that the disease decreased juice extraction, the percentage of solids and sucrose in the juice, lowered the purity, and resulted in other deleterious chemical changes. The value of the cane was materially reduced by the borer alone, but to a greater extent by the combination of borer and red rot, which are usually associated in injury to mill cane. The chemical changes in the very susceptible P. O. J. 213 cane were greater than in the susceptible P. O. J. 36–M and the resistant Co. 281.

Bourne (*25*) reported an epidemic of red rot in P. O. J. 2714 in southern Florida in 1934, which caused a 30-percent loss in tonnage on 200 acres and a reduction of approximately 50 percent in the sucrose of the harvested cane. The severity of the disease on P. O. J. 2714 resulted in its rapid replacement with more resistant varieties.

Since both P. O. J. 36–M and P. O. J. 213 have ceased to be important commercial varieties in the sugar-producing sections of Louisiana, and P. O. J. 2714 in southern Florida, red rot losses to mill cane have been much less in the United States in recent years than formerly.

[7] Estimates based on the writer's examination of approximately 500 acres of P. O. J. 36–M on 13 plantations.

While all varieties are affected to some extent every year, particularly those that are subject to heavy borer infestation, the present leading commercial varieties are more resistant to the spread of red rot in the growing stalk than those formerly grown. C. P. 807, which is very susceptible to the spread of red rot in the semidormant seed cuttings, seems to possess a degree of resistance to the disease in the growing plant, which, coupled with its resistance to borer attack, renders the disease of little importance in mill cane. The same is true of Co. 290. Of the present important commercial varieties in Louisiana, C. P. 29/116 and 29/320 are the only ones in which the disease spreads to any considerable extent in standing cane.

HISTORY OF THE DISEASE AND GEOGRAPHIC DISTRIBUTION

Red rot was first reported as a disease of sugarcane by Went in Java in 1893 (*73*). He described in detail the symptoms of the disease, proved the parasitism of the organism he isolated from the diseased tissues, calling it *Colletotrichum falcatum* Went, and carried out life-history studies. He analyzed healthy and diseased mature stalks of cane, finding that the disease caused great losses in sucrose content of mill cane. He proposed "red smut" as a common name for the disease.

Shortly after Went's description, Massee (*47*) isolated *Colletotrichum falcatum* from specimens of cane received at Kew Gardens from the West Indies. He considered it the cause of root disease of sugarcane, attributing the stalk rotting, then so destructive in the Indies, to a new fungus that he later described as *Trichosphaeria sacchari* Mass. This led to considerable confusion regarding the identity of red rot in the West Indies, which continued for a decade. Went (*74*) later compared the Javan and West Indian forms of the fungus, concluding it was highly improbable that the West Indian fungus was the cause of root rot. Howard (*41*) in 1903 finally ended the confusion regarding the etiology of the destructive disease in the West Indies commonly called "rind disease," showing that it was in reality red rot. He found that invasion by *Melanconium sacchari* Mass. (the rind disease fungus) was secondary, following severe injury by *C. falcatum*.

Within a decade after Went's description of the disease in Java, its occurrence was reported in several other countries, and from the published accounts it appears probable that it was widely distributed and well established before it was finally recognized as a new disease of sugarcane. Tryon (*68*) suggested that what had been described as a severe rotting of cane in Queensland as early as 1895 was probably red rot. Barber's early accounts (*22*, *23*) indicated that it was a very important sugarcane disease in Madras. He found that it was severe in regions where borers were almost entirely absent. He was the first to note that the thin varieties of cane are more resistant to red rot than the noble varieties. He expressed the belief that the apparent immunity of certain varieties could be broken down by bad cultivation, and found that the disease was favored by poor drainage. His observations on the influence of poor drainage and cultural conditions in favoring red rot have been verified by later workers in every country where the disease is important, and have been of basic consideration in seeking to control the disease.

Butler (*27*) conducted extensive studies of the disease in India (Bombay), devoting particular attention to the causal organism and to modes of infection. He proposed the common name of "red rot," a name now universally accepted, in preference to Went's "red smut." He noted that the fungus was parasitic on the leaves as well as in the stalks. Went had considered the leaf form a saprophyte. In accordance with Raciborski's work in Java (*53*), Butler ascribed most of the infection of growing plants to direct mycelial connection between the stalks and diseased cuttings. Entrance through the tunnels of boring insects was noted as a possible source of infection, but it was not believed to be important. He analyzed diseased and healthy stalks and found that red rot greatly reduced the sucrose content and purity of the juice. He concluded that this loss was due to the great inverting action of the fungus and not to its consumption of sucrose.

Lewton-Brain (*46*) conducted cultural studies of *Colletotrichum falcatum* in Hawaii, where he stated the disease was of minor importance. Contrary to Butler's findings in India, he concluded that borer tunnels provided the principal means of infection of growing cane.

Edgerton was the first definitely to identify and study the disease in the United States (*31*, *32*, *33*). He found that natural infection in Louisiana occurred principally through borer holes, but mentioned that in material received from Georgia, infection had occurred at the nodes without borer injury, a situation which is still true today. He stated that losses from the disease were due to reductions in stands of cane and in the inversion of sucrose in mill cane. He was unable to confirm Butler's findings that a direct mycelial connection existed between the seed cuttings and growing plants. Edgerton and Moreland (*36*) later suggested that the divergent results in this respect might be due to differences in the strains of *C. falcatum* present in the two countries, to differences in environmental conditions, or to varietal reaction to the disease. South and Dunlop (*1*) also concluded from their experiments in the West Indies that the disease was not communicated to growing stalks by infected cuttings. Kulkarni (*44*), meanwhile, had confirmed Butler's results in India.

Because of these negative results obtained in the Western Hemisphere, Butler and Hafiz Khan (*30, p. 159*) reviewed in greater detail Butler's earlier experiments (with Red, Purple, and Ashy Mauritius canes), which had led to such a conclusion, and stated that—"The course of the infection up into the stem can be traced in many cases and direct connection between the mycelium in the sett and that in the new shoot established." They also found that infection occurs through the root primordia. They were unable to produce infection by inoculation of the leaf scars or the uninjured buds. They proved by inoculation experiments that the forms of the fungus occurring on the leaves and in the stalks were equally parasitic and identical. The use of disease-free seed cuttings was emphasized as the most important means of controlling the disease, which they stated was the greatest obstacle to successful sugarcane cultivation in many parts of India.

That red rot was one of the most widespread (or at least widely recognized) sugarcane diseases during the early years of the present century is attested by the numerous references to its economic importance. Reports of its occurrence in the West Indies included those of South (*63*, *64*), Ballou (*21, pp. 332–342*), Nowell (*51*), Johnston

(*42*), and Johnston and Stevenson (*43*). The latter authors found that the losses due to the disease in Puerto Rico in no way compared in severity with those in other countries. Averna Saccá (*20*), in Brazil, and Stockdale (*66*), in Mauritius, added to the known geographic distribution of the disease.

During the past 20 years, red rot has received relatively little attention in the world's sugarcane pathology literature compared with the first 25 years of its recognition as a disease. This was probably due in part to the fact that it was no longer new, its life history and importance having been fairly well cataloged in most of the sugarcane countries. Perhaps of greater importance, however, was the fact that it had actually become less destructive because of the changes to more resistant varieties that had occurred in many countries. While red rot itself was responsible for some of these changes (*22, 30, 44, 52*), other diseases played a more important role in certain countries. The gradual replacement of the noble varieties with those of hybrid origin over the entire sugarcane world has undoubtedly had a great influence in diminishing the losses due to red rot. According to Shepherd (*62*), red rot has ceased to be a serious disease in Mauritius, as the result of the cultivation of resistant varieties.

Recent studies of the disease have been confined largely to the United States, where red rot is the principal cause of seed deterioration. Its importance as a disease of seed cane has been considered by Edgerton and Moreland (*36*), Edgerton (*34*), Edgerton as quoted in The Sugar Bulletin (*3, 4*), Edgerton and Flor (*35*), Edgerton, Taggart, and Tims (*37*), Edgerton and Tims (*38*), Tims and Edgerton (*67*), and Abbott (*8, 9, 11*). Edgerton, Tims, and Mills (*39*) described it as one of the factors causing stubble deterioration of sugarcane in Louisiana.

Roldan and Tecson (*58*) stated that red rot is a common but not a serious disease in the Philippine Islands. Abbott (*7*) reported a similar situation in Peru.

This account of the progress in the knowledge of red rot throws some light on the origin of the disease. That it originated in the Eastern Hemisphere seems probable in view of its known widespread distribution and the destruction it was causing in Eastern countries shortly after its recognition as a disease. At the same time, however, it was epidemic in the West Indies, assuming, as Howard has shown, that the so-called "rind" and "root" diseases were in reality red rot. But it seems logical to assume that the wider distribution in the Eastern countries was the result of longer establishment in them. Furthermore, if originally a parasite of sugarcane, it would presumably be more widespread where this plant had long been established than in countries where sugarcane was of more recent introduction. The time and manner of its spread over the world are not definitely known, but presumably it was introduced into new regions along with cuttings of sugarcane transported for seed.

It is apparent from the literature review that red rot is a destructive disease primarily in subtropical countries. This is particularly true insofar as it relates to destruction of seed cane. In the tropical countries, where higher temperatures cause the seed cuttings to germinate soon after planting and to continue growing without a period

of retardation, the disease has little opportunity to develop and to injure the seed cane. In subtropical countries, on the other hand, the seed cuttings may remain in the soil for several weeks after planting before active growth can begin, during which time red rot may develop and severely rot the seed cane. Environmental conditions thus appear to be more favorable to the disease in subtropical countries, but the possibility should not be overlooked that differences in parasitic races of the causal organism present in various countries may also be concerned.

FAILURE OF P. O. J. 213 IN LOUISIANA

When the red rot project was undertaken at the United States Sugar Plant Field Station at Houma, La., in the summer of 1930, one of the primary objects was to determine the reason for the failure of the commercial variety P. O. J. 213 from this disease in Louisiana, while in the sirup-producing States, where it had been grown and exposed to red rot for an equal number of years, it had shown no indication of injury. As mentioned earlier, it had become one of the leading commercial varieties in Louisiana following the replacement of the old noble canes beginning in 1926, and at the time of its release for commercial cultivation, and for sometime thereafter, published reports indicated that it was resistant to red rot (*2, 3, 34*). It is not clear, however, whether the evidence of its resistance was obtained from controlled inoculation experiments or from observations of its reaction to the disease in the field in comparison with the old varieties. If its classification as a resistant variety was based only on field observations, it is possible that it may have merely escaped infection and its inherent susceptibility thus escaped detection. But Tims and Edgerton (*67, p. 4*) state that—"When this variety was first introduced into the State there was very little red rot infection found, even though there was very heavy borer damage and other conditions were apparently favorable for the disease to develop."

The records show that it gave satisfactory stands and yields on both light and heavy soils from 1926 to 1929 (*17, 18, 57*) and passed through at least 1 year, 1927, when red rot was severe on the old varieties, with only slight injury by the disease (*34*). The first indication of poor germination by the variety was obtained in an experiment by Flor (*40*) in 1928–29, who observed that it gave only 13.5-percent germination in a test on heavy soil. This was probably due to red rot, although the author did not so state.

In the spring of 1930, many fields of both plant and stubble cane failed to germinate, and examination of the seed cuttings and the stubbles revealed extensive injury by red rot.[8] Stands on thousands of acres were complete failures, while the gappy stands on many others not only greatly increased cultivation costs per ton of cane harvested but doubtless also lowered the quality of the juice. This experience was repeated in 1931 and to a considerable extent in succeeding years, until by 1934 P. O. J. 213 had ceased to be an important commercial variety in the Sugar Belt of Louisiana.

[8] Unpublished notes by R. D. Rands.

POSSIBLE CAUSES OF THE FAILURE

Four possible causes for this failure have been suggested (*11*):

(1) An actual change in the inherent resistance of the variety, which would presume an en masse change in its genetic constitution and is therefore hardly to be considered tenable.

(2) An increase in susceptibility as the result of long-continued subjection to unfavorable environmental conditions, resulting in nutritional or physiological disturbances. This seems improbable in view of the fact that cuttings of P. O. J. 213 from the muck lands of southern Florida, the sandy lands of Georgia and Mississippi, and the various soil types of the Lousiana sugar belt have all proved equally susceptible when artificially inoculated.

(3) The possibility that, prior to 1930, the variety had not been subjected to conditions favorable to the development of red rot, * * *. [This appeared to be refuted by the experience of the red rot epidemic in 1927, already referred to.]

(4) The development of a strain of the red-rot fungus more virulent on P. O. J. 213 than those present in the State prior to its introduction, or of a virulent one previously present which had not found conditions favorable for its development.

Since the first three possibilities did not provide an adequate explanation of the failure, attention was directed toward a study of the biology of the fungus itself as offering the greatest likelihood of a solution. Aside from the general interest in explaining the failure of this one variety, an understanding of the underlying principles involved was of great importance in the breeding and varietal-testing programs, if such a situation were to be avoided in the future with other initially resistant varieties.

The history of varietal changes in Louisiana did not strongly support the theory of a change in the red rot flora as being important in such changes, although there was some indication that the sudden increase of a very virulent strain of the fungus might have been concerned in the final failure of Louisiana Purple. The views of Tims and Edgerton (*67, p. 4*) are of interest in this connection:

The way in which the red-rot disease develops in certain years leads one to suspect the sudden appearance of new highly parasitic strains of the fungus. The Purple cane was grown successfully in Louisiana for many years after it was first described as being quite susceptible to red rot. The disease always developed to a greater or less extent but never ruined the crop. However, in the last year this variety was grown commercially in the State, there was the worst development of red rot that had ever been recorded. A plot of several acres of Purple cane in the experiment station at Baton Rouge on the first of September 1927 was estimated to yield from 35 to 40 tons per acre. A short time later red rot began to develop very rapidly. When the cane was cut for the mill in early November practically every stalk in the field was infected. Many stalks had completely decayed and the rind had shriveled. Such a development of red rot is hard to explain except by assuming that a highly parasitic strain of the fungus spread over the fields that year.

At the time of the failure of the noble canes, Purple and Ribbon had been successfully grown in Louisiana for over a century, during perhaps 25 years of which they had been exposed to red rot. D–74, which was introduced in 1891, although not greatly extended on many plantations until 1904–5, had been exposed to the disease for an equal period. If specialized forms of *Colletotrichum falcatum* were principally responsible for their failure, it seems reasonable to assume that it would not have required a quarter of a century to bring about such a result. There is a possibility, of course, that a new strain of the fungus was introduced into the State prior to the institution of the two national quarantines (*69, 70*) on sugarcane in 1914. However, the records show that the decline of the noble varieties was gradual

over a period of years, in contrast with the sudden collapse of P. O. J. 213, and while the failure of the old varieties is now generally accepted to have been due to a combination of mosaic, root rot, and red rot, it was not until mosaic had become widespread over the State that they finally failed. There is no clear-cut evidence, therefore, that specialized races of *C. falcatum* were responsible for the failure of the noble varieties.

With respect to P. O. J. 213, however, the evidence is indisputable that its collapse was due to red rot, and strongly indicated that specialized races of *Colletotrichum falcatum* were concerned. It thus seemed probable that some phenomenon not previously experienced in Louisiana was principally concerned in the failure of P. O. J. 213, and it was clear that some factor was operating in that State which had not affected the variety in the sirup-producing States. A survey of the red rot flora of the southern United States was undertaken, therefore, as the first step in the study of the problem.

SURVEY OF THE RED ROT FLORA

METHOD OF SAMPLING AND ISOLATION

Isolations of *Colletotrichum falcatum* were made from both leaf and stalk lesions of the standard varieties in plantation and farm fields, and from a number of seedlings in the pathological nurseries and agronomic test fields. A standard procedure was followed in obtaining the samples and in making the isolations. Samples of leaf midribs were collected by taking from 10 to 25 leaves at random at scattered points in plantation fields. If possible, they were taken to the laboratory for culturing the same day, but frequently several days elapsed between the time of collection and culturing. In such cases the samples were kept in a fresh condition by wrapping in oiled paper. A section of the midrib lesion about one-half inch long was cut from each leaf collected, washed in sterile water, treated from 2 to 3 minutes with an aqueous solution of mercuric chloride, 1:1,000, washed three times in sterile water, drained, and plated in oatmeal agar acidified with 1 drop of 50-percent lactic acid per 25 cc of medium. Sections of tissue cut from stalk lesions were cultured in the same manner. The oatmeal agar was prepared by steaming 100 g of rolled oats in 1 liter of water for an hour, filtering through cheesecloth, adding 1-percent agar to the filtrate, and autoclaving at 15 pounds pressure for 15 minutes.

GEOGRAPHIC DISTRICTS

For the purposes of the survey, the sugar section of Louisiana was divided into three geographical districts: The southeastern parishes, comprising Terrebonne, Lafourche, and those along the lower Mississippi River; the northern parishes, comprising Iberville, West Baton Rouge, Avoyelles, Pointe Coupee, and St. Landry Parishes; and the western parishes, comprising those west of the Atchafalaya River and south of St. Landry Parish.

As the opportunity arose, similar collections were made in the sirup-producing sections of Mississippi, Alabama, Georgia, and northern Florida. Most of the samples for survey in these States were collected on annual trips during the fall months, but consider-

able material from Georgia and Mississippi was collected by B. A. Belcher.

The isolations from southern Florida were made from the sugar-producing district around Lake Okeechobee, except for a few cultures obtained in 1936 from sirup patches near Sarasota.

RESULTS OF THE SURVEY

DISCOVERY OF TWO MORPHOLOGICAL RACES OF COLLETOTRICHUM FALCATUM

The survey was first undertaken in Louisiana, where more than 1,000 isolates were obtained in 1930 from localities in all parts of the sugar section of the State. These were all morphologically uniform in culture. On oatmeal agar they produced a floccose, white to light-gray turf, usually with pink slimy masses of conidia appearing abundantly over the surface in 2 to 3 weeks. While slight variations in texture, color, and abundance of fruiting were noted, they were not great enough to suggest any grouping of the isolates on a morphological basis.

In the late fall of 1930, a trip was made to the sirup-producing section of southern Georgia and northern Florida, where additional isolations were made. A survey of the red rot flora of these States was deemed of particular importance since, as previously mentioned, P. O. J. 213 had been grown there almost as long as in Louisiana, but had shown no indication of red rot injury. It was felt that a comparison of the forms of *Colletotrichum falcatum* from Louisiana, where P. O. J. 213 was failing, with those in the sirup States, where it was still being successfully grown, might have an important bearing on explaining the variety's behavior in Louisiana.

One hundred isolates were obtained from the sirup States, and on examining them in culture it was immediately apparent that they were strikingly different in color and texture of the turf from the Louisiana isolates. They were dark gray in color and of a velvety, compact texture, in contrast to the light color and floccose, cottony texture of the Louisiana forms. The superficial pink masses of conidia were lacking. By means of inoculation experiments it was proved that both the Louisiana forms and those of the sirup States produced typical red rot in sugarcane stalks and leaves.

This was the first evidence of the existence of two morphological groups of *Colletotrichum falcatum*. The groups were readily distinguishable in culture on oatmeal agar, yet the various isolates of each were sufficiently uniform among themselves that no further subdivision on a morphological basis was suggested. That one group should occur exclusively or at least be predominant in one State where P. O. J. 213 was failing from red rot, and another group predominate in States where it had shown no indication of such injury, was considered extremely significant, although it was recognized that differences in soils, climate, cropping conditions, or moth borer infestation might be determining factors in the differences in red rot injury sustained by P. O. J. 213 in the two areas. The situation suggested, however, that the failure of P. O. J. 213 in Louisiana had been due to a change from an older and possibly less virulent red rot flora (dark types) to the now dominant and possibly more virulent

(on P. O. J. 213) light types. The discovery of the two groups gave added importance to the survey and suggested the desirability of attempting to obtain a connecting link between the red rot floras prior and subsequent to the introduction of the P. O. J. varieties.

RELATION OF THE TWO GROUPS TO THE OLDER RED ROT FLORA IN LOUISIANA

Unfortunately, isolates of *Colletotrichum falcatum* from Louisiana prior to the time of the introduction of P. O. J. 213 were not available for study. In lieu of such cultures, it seemed possible that the hypothetical older flora might be recovered from sections of Louisiana where the noble canes were still cultivated and the P. O. J. varieties not yet introduced. The only large district where such a situation might exist appeared to be the sirup-producing parishes of northern Louisiana, located from 75 to 100 miles north of the sugar belt of the State, and where the noble varieties had been grown for many years without any considerable contact with outside sources of seed supply. Accordingly, a trip was made to this district in the fall of 1931.

Unfortunately for the purposes of the survey, Purple and Ribbon, then the principal sirup canes of northern Louisiana, rarely have leaf midrib lesions, and the absence or rare occurrence of the moth borer made it improbable that red rot would be obtained from standing cane. Many patches of these varieties were examined in De Soto, Caddo, Bossier, Webster, Lincoln, Jackson, Winn, Ouachita, and Caldwell Parishes without obtaining any specimens of red rot. Nor were midrib lesions found in the few scattered patches of Cayana encountered. A second trip was made to these parishes in the spring of 1932 in the hope that red rot might be obtained from bedded seed cane, but no specimens of the disease were obtained. Farmers stated that their bedded seed cane always kept perfectly, except for occasional "dry rot" (drying out), and that they had never seen any disease in their cane resembling the symptoms of red rot described to them. It was therefore concluded that red rot is absent or very rare in the sirup-producing sections of the State, making it improbable that the older red rot flora could be obtained from there.

Another locality where the P. O. J. varieties were not grown was on Bayou DuLarge, about 20 miles south of Houma, La. There a sirup enterprise is conducted with an assortment of noble canes, among them Purple, Ribbon, D–74, and Cristalina, and while there was no proof that red rot contamination from the fields of P. O. J. varieties 10 to 15 miles distant had not occurred, the farmers said these had not been grown in the immediate vicinity to their knowledge. Red rot is prevalent in this sirup district, and in the fall of 1931 *Colletotrichum falcatum* was isolated from stalks of D–74, Purple, and Ribbon. The isolates obtained were all light types. Again in 1932 light types were obtained, but in 1933 one dark type was isolated from D–74, which in a comparative virulence test (see test F, p. 51) proved to be less virulent on P. O. J. 213 than the light types from the latter variety. Therefore, while some circumstantial evidence favored the hypothetical existence of an earlier dominant dark type population of the fungus, no sizable remnants were found to substantiate it.

Since the survey did not antedate 1930, no direct evidence is available demonstrating a change in the red rot flora that may have occurred in Louisiana between 1924, the time of the introduction to commercial culture of the P. O. J. varieties, and 1930 when signs of failure of P. O. J. 213 were first noted. Since 1930, however, the survey has provided definite data linking changes in the red rot flora with wide-scale changes in acreage and kinds of sugarcane varieties grown.

The results of the survey suggest two possible explanations for the anomalous behavior of P. O. J. 213 prior and subsequent to 1930. It may reasonably be assumed from the evidence that both morphological races of *Colletotrichum falcatum* were present in the State prior to the introduction of the P. O. J. varieties into Louisiana. The strict quarantine measures adopted by the United States Department of Agriculture, which is the sole agency permitted to import foreign varieties, make it highly improbable, if not impossible, that red rot was carried in the original seed cuttings of the P. O. J. canes introduced into Louisiana. Assuming then that both morphological races were present in the State at the time of this introduction, two possible explanations for subsequent events may be offered:

First, that the dark races were dominant on the noble varieties, and it was only after several years of the cultivation of P. O. J. 213, which is apparently particularly susceptible to the light races, that certain parasitic races of the latter multiplied on it and eventually caused its failure.

Second, that the light races were dominant on the old varieties when P. O. J. 213 was introduced, but that certain parasitic races among them proved much more destructive to P. O. J. 213 than they had to the old varieties. The evidence favors the first alternative, since dark races have not been found causing injury to P. O. J. 213 in Louisiana and in the sirup-producing States where, prior to 1937, the variety had been exposed to infection by the dark races to the practical exclusion of the light ones, it had not suffered from red rot.

CHANGE IN DOMINANT RED ROT POPULATION IN LOUISIANA IN RELATION TO CHANGE IN VARIETIES

In table 1 the number of isolates of *Colletotrichum falcatum* obtained from 1930–37 from fields on representative plantations in every parish of the sugar district of Louisiana, from farm fields in the sirup-producing States, and from southern Florida are summarized according to host variety. It will be noted that all of the 1,029 isolates obtained in Louisiana in 1930 were light races. In 1931, two dark-race isolates were obtained. One was from a leaf lesion on Cayana from what was probably the last remaining commercial field of the variety in Terrebonne Parish (Hollywood Plantation), and the other from rotted seed cane of C. P. 807 (Ranch Plantation). In 1932 and 1933, dark-race isolates were obtained from leaves of Co. 290 in Lafayette Parish (Billeaud Plantation) and in 1933 from rotted banked seed cane of P. O. J. 234 in Terrebonne Parish (Woodlawn Plantation). In 1934, a very definite change in the relative proportions of the two races became apparent (fig. 3). Cultures made from Co. 281, Co. 290, and P. O. J. 36-M from the same plantations, where only light races had been ob-

tained from the same varieties in previous years, began to show a high percentage of dark races. Those from C. P. 807 were also predominantly dark, and of particular significance is the fact that this increase in dark races coincided with the first important red rot germination injury to the variety. In 1934 an equal percentage of light- and dark-race isolates was obtained from all varieties, in 1935 there was a slight predominance of the light race, while in 1936 and 1937 there was a decided increase in the relative numbers of the dark-race isolates, as may be seen in table 1 and figure 3.

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TABLE 1.—*Number of light- and dark-race isolates of Colletotrichum falcatum obtained from various varieties of sugarcane in the southern United States*

Variety	1930		1931		1932		1933		1934		1935		1936		1937		Total			
																	Light		Dark	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Number	Percent	Number	Percent
Louisiana:																				
Southwestern parishes:																				
C. P. 807	23	0	4	1	4	0	...	...	1	11	2	31	0	22	8	75	42	23.1	140	76.9
C. P. 28/19	...	...	4	0	...	...	...	...	10	13	7	20	15	18	9	90	45	24.2	141	75.8
Cayana	...	...	10	1	15	0	...	...	...	...	...	...	...	...	...	...	25	96.2	1	3.8
Co. 281	56	0	10	0	23	0	42	0	11	16	19	43	11	4	21	90	193	55.8	153	44.2
Co. 290	34	0	...	...	...	...	10	0	...	...	2	14	...	...	22	54	68	50.0	68	50.0
D-74	137	0	10	0	8	0	20	1	...	...	3	0	...	...	...	...	178	99.4	1	.6
P. O. J. 36-M	127	0	5	0	...	...	...	...	...	...	12	11	...	...	8	35	152	76.8	46	23.2
P. O. J. 213	63	0	50	0	45	0	12	0	20	1	36	7	...	...	20	9	246	93.5	17	6.5
P. O. J. 234	28	0	9	0	2	0	0	10	...	...	18	6	...	...	...	...	57	78.1	16	21.9
Miscellaneous varieties	236	0	72	0	4	0	4	0	...	...	...	...	...	...	7	33	323	90.7	33	9.3
Total	704	0	174	2	101	0	88	11	42	41	99	132	26	44	95	386	1,329	...	616	...
Percent	100	0	98.9	1.1	100	0	88.9	11.1	50.6	49.4	42.9	57.1	37.1	62.9	19.8	80.2	...	68.3	...	31.7
Northern and western parishes:																				
C. P. 807	2	0	...	...	2	0	...	...	2	8	0	6	4	1	6	1	16	50.0	16	50.0
C. P. 28/19	...	...	...	...	...	...	...	...	41	66	14	5	14	26	3	10	72	40.2	107	59.8
Co. 281	15	0	8	0	16	0	18	0	55	82	7	25	0	1	8	28	127	48.3	136	51.7
Co. 290	...	...	...	...	14	8	20	8	25	25	...	...	...	...	17	14	76	58.0	55	42.0
P. O. J. 36-M	...	...	...	...	39	0	24	0	57	48	10	13	...	...	...	...	130	68.1	61	31.9
P. O. J. 213	172	0	30	0	14	0	8	0	14	2	54	0	...	...	12	6	304	97.4	8	2.6
P. O. J. 234	61	0	...	...	8	0	...	...	29	0	16	0	...	...	12	0	116	100.0	0	0
Miscellaneous varieties	75	0	...	...	8	0	4	2	21	14	...	...	1	6	6	23	115	71.9	45	28.1
Total	325	0	38	0	101	8	74	10	244	245	101	49	19	34	54	82	956	...	428	...
Percent	100	0	100	0	92.7	7.3	88.1	11.9	49.9	50.1	67.3	32.7	35.8	64.2	39.7	60.3	...	69.1	...	30.9
Grand total	1,029	0	212	2	202	8	162	21	286	286	200	181	45	78	149	468	2,285	...	1,044	...
Percent	100	0	99.1	0.9	96.2	3.8	88.5	11.5	50.0	50.0	52.5	47.5	36.6	63.4	24.1	75.9	...	68.6	...	31.4

Sirup-producing States:																				
C. P. 807	0	10			0	5					0	5					0		20	100.0
Cayana	0	40	0	112	0	46	0	18	0	34	1	132	0	14	0	12	1	0.2	408	99.8
Co. 281			0	8							4	14					4	15.4	22	84.6
Co. 290									0	12	3	14	2	0			5	16.1	26	83.9
Louisiana Purple	0	1	0	7					14	0	9	0	1	5			24	64.9	13	35.1
P. O. J. 213	0	12	0	8	0	16					6	38			0	1	6	7.4	75	92.6
Miscellaneous varieties	0	6	0	8	0	5	0	3			7	15	3	1			10	20.8	38	79.2
Total	0	69	0	143	0	72	0	21	14	46	30	218	6	20	0	13	50		602	
Percent	0	100	0	100	0	100	0	100	23.3	76.7	12.1	87.9	23.1	76.9	0	100		7.7		92.3
Southern Florida:																				
Co. 281			8	0	12	0							7	0			27		0	0.0
P. O. J. 213			10	0	8	0							4	0			22		0	0.0
P. O. J. 2714					10	0			12	0			5	0			27		0	0.0
Miscellaneous varieties			12	0	6	0			16	0			18	0			52		0	0.0
Total			30	0	36	0			28	0			34	0			128		0	0.0
Percent			100	0	100	0			100	0			100	0				100.0		0.0

This change took place coincident with the rapid expansion in acreage of Co. 281, Co. 290, C. P. 807, and C. P. 28/19, and the corresponding decline of the P. O. J. varieties. Since Co. 281, Co. 290, and C. P. 28/19 generally exhibit a much higher percentage of leaf infection in the field than P. O. J. 213 and P. O. J. 234, which had hitherto occupied a major portion of the acreage in Louisiana, one would expect that they would exert a considerable influence on the multiplication and dissemination of races of *Colletotrichum falcatum* to which they are susceptible. And since the evidence indicates that they favor the dark races, it seems probable that the great increase in relative occurrence of this morphological race beginning in 1934

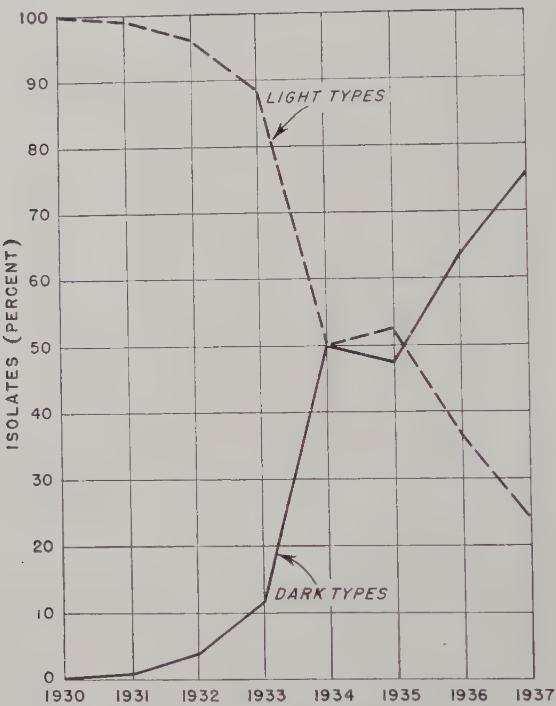


FIGURE 3.—Percentage of light- and dark-type isolates of *Colletotrichum falcatum* obtained from sugarcane in southern Louisiana from 1930–37. (Data from table 1.)

resulted from the expansion of these varieties. It is true that the isolations from P. O. J. 36–M, which then occupied a negligible acreage, likewise showed an increase in the dark races in 1934 and 1935 (table 1) from the same localities where only the light ones had formerly been obtained, but it is possible that this resulted from mass spread of the dark races to 36–M from the more favorable hosts (Co. 281, Co. 290, and C. P. 807) on which they greatly multiplied. Too much dependence, of course, cannot be placed on the relative percentages of the light and dark races for the sugar district as a whole, since unequal numbers were obtained from different varieties and from different localities. Nevertheless, the data show definitely that a change in the relative proportion of the two morphological groups occurred coinci-

dent with the change in sugarcane varieties, and indicate that there was a relationship between the two.

The absence of the dark races in the 1930 survey, which was equally as comprehensive as those of other years, and the isolation of them only occasionally until 1934, leads to speculation as to the origin of the dark races when they finally appeared. It is entirely possible that they were present in the fields of the P. O. J. varieties during these years, but in such small relative proportions that a more extensive survey would have been necessary to discover them. There is a further possibility that they were harbored on Johnson grass. This grass is commonly present in or near fields of sugarcane throughout the sugar district of the State, and while *Colletotrichum falcatum* occurs less abundantly on it than on sugarcane, it can usually be found in the late summer or fall in any fairly large group of this grass (plate 2, *E*). Isolations of *C. falcatum* from leaf midrib lesions on it have yielded the dark races about two and a half times as frequently as the light ones. Whether the fungus is able to exist on this grass from one growing season to the next, or whether the infections on it originate each year from sugarcane, has not been determined. It is within the realm of possibility, however, that Johnson grass may be capable of harboring either morphological or parasitic races of the red rot fungus, which might later become widely disseminated on a congenial sugarcane host.

ASSOCIATION OF MORPHOLOGICAL RACES OF COLLETOTRICHUM FALCATUM WITH CERTAIN SUGARCANE VARIETIES

The survey provided evidence of the association of certain morphological races of *Colletotrichum falcatum* with severe red rot injury to at least two sugarcane varieties in Louisiana, P. O. J. 213 and C. P. 807. The 394 isolates obtained from P. O. J. 213 on 25 representative plantations in all parts of the sugarcane district of the State during the years 1930–33, which covered the period of its commercial decline, were all the light-colored morphological types. While this may not necessarily indicate a preference of P. O. J. 213 for the light races as compared with the dark, since few of the dark races were obtained in Louisiana from other commercial varieties during the same period, it does not alter the fact that the light races were predominant on the variety during the period of its decline, and were apparently responsible for it. No dark races were obtained from P. O. J. 213 until 1934, by which time only scattered fields of the variety remained in commercial cultivation in the Sugar Belt of Louisiana. Of the 10 dark races obtained from it in 1934 and 1935, only 1 was isolated from a stalk lesion, the others having come from leaves. The light races continued to predominate on this variety after 1934 in spite of the fact that it was probably equally exposed to infection by the dark races, since they greatly increased on other varieties during this period. On Raceland Plantation (Lafourche Parish) in 1935, for example, a small field of P. O. J. 213 stubble yielded only light races from red rot lesions in the stalks, and a ratio of 4 light to 1 dark from the leaves. In an adjoining area of Co. 281 the ratio was 3 to 1 in favor of the dark races. On Ridgland Plantation (Terrebonne Parish) in the same year, stalks of P. O. J. 213 stubble yielded only light races, the leaves a ratio of 3 light to 1 dark, while in an adjacent square of Co. 281 the ratio was 2 to 1 in favor of the dark. A small field of

stubble on a farm near Gibson, La. yielded only light races from stalk lesions, while both races were obtained from leaves of Co. 281 adjoining it. In 1937, only light races were obtained from P. O. J. 213 stalks from a 5-acre field in St. Mary Parish, and only dark races from Co. 281 surrounding it. These examples indicate greater susceptibility of P. O. J. 213 to the light races under conditions of exposure to natural infection by both.

From C. P. 807, on the other hand, 91 percent of the isolates obtained during the years 1934–36, when red rot injury to the variety was severe on many plantations, were dark races, and these forms were obtained exclusively from areas where the disease was most severe. Since during this same period both dark and light races were obtained from Co. 281, Co. 290, P. O. J. 36–M, and P. O. J. 213 on the same plantations and sometimes from adjacent fields, the C. P. 807 was presumably exposed to infection by both morphological races. On Crescent Farm Plantation (Terrebonne Parish), for example, in fields adjacent to or not far distant from the C. P. 807, where only dark races were obtained in 1935, 4 light and 4 dark races were isolated from Co. 281, 3 light and 15 dark from C. P. 28/19, and 9 light and 10 dark from P. O. J. 36–M. On Greenwood Plantation (Lafourche Parish), where only dark races were obtained from C. P. 807 in that year, 2 light and 14 dark races were isolated from Co. 290 in adjoining fields, 3 light and 13 dark from Co. 281, and 3 light and 1 dark from P. O. J. 36–M. On Raceland Plantation (Lafourche Parish), where only dark races were obtained from C. P. 807, 18 light and 6 dark races were isolated from P. O. J. 234 in an adjacent field, 6 light and 16 dark from Co. 281, and 13 light and 3 dark from P. O. J. 213 not far distant. In 1936 on Crescent Farm Plantation, only light races were isolated from C. P. 28/19 in the same heavy soil area where only dark races were isolated from C. P. 807. On Greenwood Plantation about equal numbers of light and dark races were obtained from C. P. 28/19 and Co. 290, the latter adjoining the C. P. 807, while only dark races were isolated from the C. P. 807. Similar results were obtained in the 1937 survey. In view of this apparent exposure to infection by both races, therefore, it seems logical to assume that the predominance of the dark races on C. P. 807 resulted from the greater susceptibility of this variety to them. That only dark races were obtained from fields where red rot injury to the variety was severe indicates that this morphological group was principally responsible for such injury.

RESULTS IN THE SIRUP-PRODUCING STATES

It will be noted from table 1 that only dark races were isolated in the sirup-producing States from 1930–33. In the spring of 1934, 10 light races were obtained from rotting, bedded seed cane of Louisiana Purple at Cairo, Ga., and later in the year, 4 additional light races were isolated from the leaves of the cane growing from this lot of seed. Light races were again obtained from rotting seed cane of this variety in 1935 and 1937.

Cayana gave dark races exclusively from commercial fields of cane throughout the 8 years included in table 1, and also in a continuance of the survey conducted in the spring of 1938. The only light race ever isolated from this variety was obtained from a leaf lesion collected in a variety test plot at Poplarville, Miss., in 1935, where it had possibly

been subjected to infection from other varieties growing in adjacent plots.

All of the isolates from P. O. J. 213 from the sirup States listed in table 1 were from leaf lesions, red rot from natural infection never having been encountered in stalks of this variety from the sirup States during the years covered by the data in this table. The rare instances of deterioration of bedded seed cane that came to attention during these years were due to *Ceratostomella paradoxa* (De Seynes) Dade, *C. adiposum* (Butler) Sartoris, or to drying out because of unfavorable bedding conditions. One light race was isolated from a field of P. O. J. 213 near Cairo, Ga., in 1935, and five additional ones from farm fields of the variety in central Mississippi in the same year.

In the spring of 1938 a distinct change in the reaction of P. O. J. 213 to red rot in southern Georgia was noted. In February, moderate red rot injury was found in beds of seed cane of this variety in Grady County, and 53 light-race isolates of *Colletotrichum falcatum*, all from nodal infection, were obtained from that number of stalks showing symptoms of the disease. By March, according to B. A. Belcher, when planting operations began, red rot had become generally severe in P. O. J. 213 seed cane throughout the southern Georgia sirup area, that on many farms being rendered entirely worthless by the disease. The situation seemed comparable to that which had occurred several years earlier, when the variety suddenly failed in Louisiana.

Whether this increase in the incidence of red rot in P. O. J. 213 resulted from environmental conditions exceptionally favorable to infection by the disease during the winter of 1937–38, or was the result of an increase in forms of the red rot fungus more specific toward P. O. J. 213, i. e., light races, has not been definitely determined. However, the evidence strongly indicates that it can be traced to a change in the predominating forms of the red rot fungus occurring in the sirup-producing areas.

During the early years of the cultivation of Co. 290 and C. P. 29/116 in the sirup States, a predominance of dark-race isolates was obtained from them. The isolations were made from small plots of the varieties growing in the vicinity of Cayana, however, and the forms occurring on them may have been influenced by proximity to the latter variety. In the spring of 1938, in isolations made from seed cane taken from larger areas of Co. 290 and C. P. 29/116, the light races predominated in the ratio of 40 to 1. These varieties now occupy approximately 10 to 15 percent of the sugarcane acreage in the sirup States and are being rapidly expanded. If, as present evidence indicates, they favor the light races of *Colletotrichum falcatum* present in these areas, they may exert an important influence on the increase in the relative proportion of this group in the general red rot population. Like Cayana, these varieties are very susceptible to leaf infection, and by virtue of this fact they may be expected to have an equally important effect on the dissemination of forms of the red rot fungus that they particularly favor. The apparent increase of the light cultural race of the fungus as the result of the cultivation of these varieties immediately suggests a connection with the increased incidence of red rot in P. O. J. 213.

It is of interest to note that the tendency for light races to increase in relative numbers in the sirup-producing States occurred at the same time that the reverse shift in population was occurring in Louisiana.

Up to 1937, however, the change had not been as great in the sirup States as in Louisiana. The greater stability of the red rot population in these States may probably be attributed to the less extensive changes in varieties that occurred there during the years of the survey. Cayana, by reason of its extreme susceptibility to leaf infection and production of such enormous numbers of conidia on the leaf lesions, doubtless continued to exert a great mass effect on the red rot population of the sirup States, in spite of the fact that it was yielding to P. O. J. 213 in acreage. P. O. J. 213 carries a very low percentage of leaf infection, and, as will be shown later, appears to be more resistant than Cayana to the strains of *Colletotrichum falcatum* occurring on the latter variety. The rather extensive increase in acreage of P. O. J. 213, which has occurred since 1930, apparently has had little effect on the dominant red rot flora. When fields of it are located at some distance from Cayana, midrib lesions are seldom found. Purple and Ribbon, both of which carry a low percentage of leaf infection and now occupy a relatively small acreage, probably have little effect on the general red rot population. As already pointed out, however, the relative proportions of light and dark races in the future may be greatly influenced by the now rapidly expanding acreages of Co. 290 and C. P. 29/116 at the expense of Cayana and P. O. J. 213.

RESULTS IN SOUTHERN FLORIDA

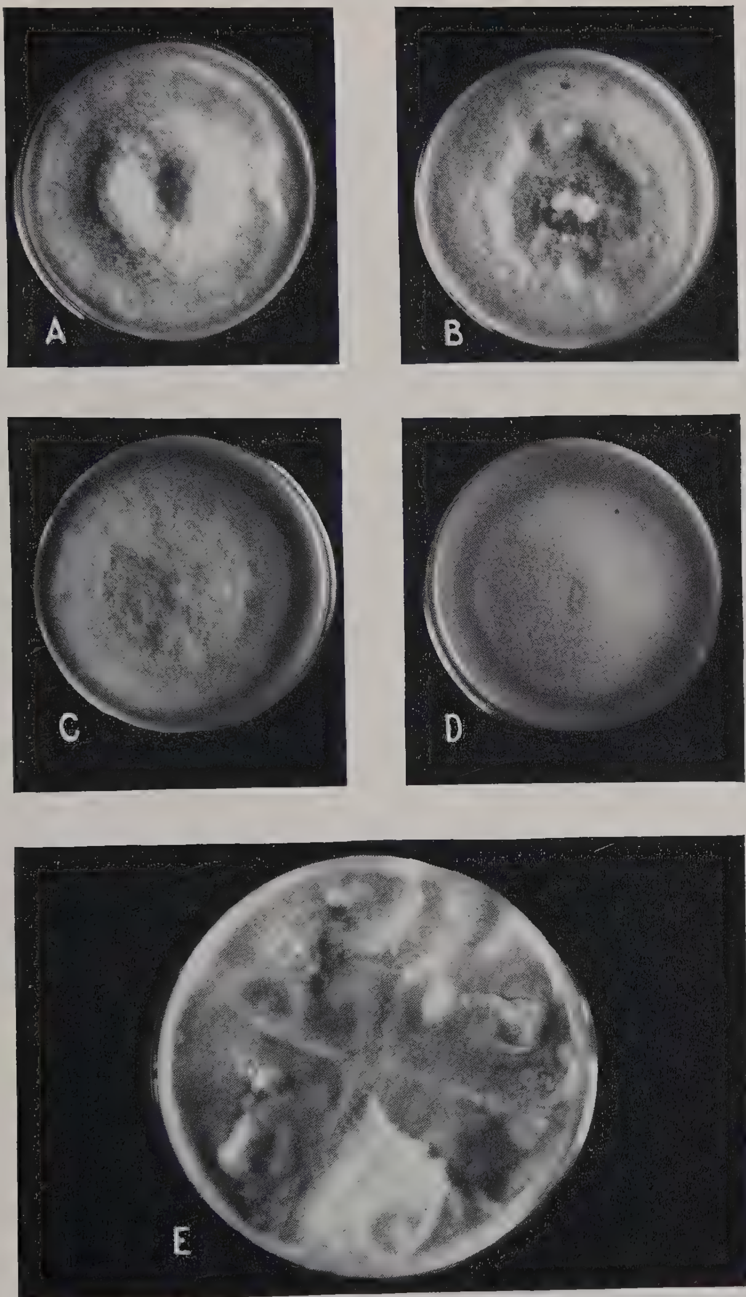
From southern Florida only light races were obtained, which were morphologically indistinguishable from the light-type isolates from the other geographic areas. In view of the differences in commercial varieties in southern Florida and the other regions, no particular significance could be attached to the occurrence of a uniform population there. P. O. J. 213 and Cayana have never been important commercial varieties in southern Florida. P. O. J. 2714 is the only commercial variety that has suffered serious damage by red rot in that section.

CULTURAL CHARACTERS OF COLLETOTRICHUM FALCATUM

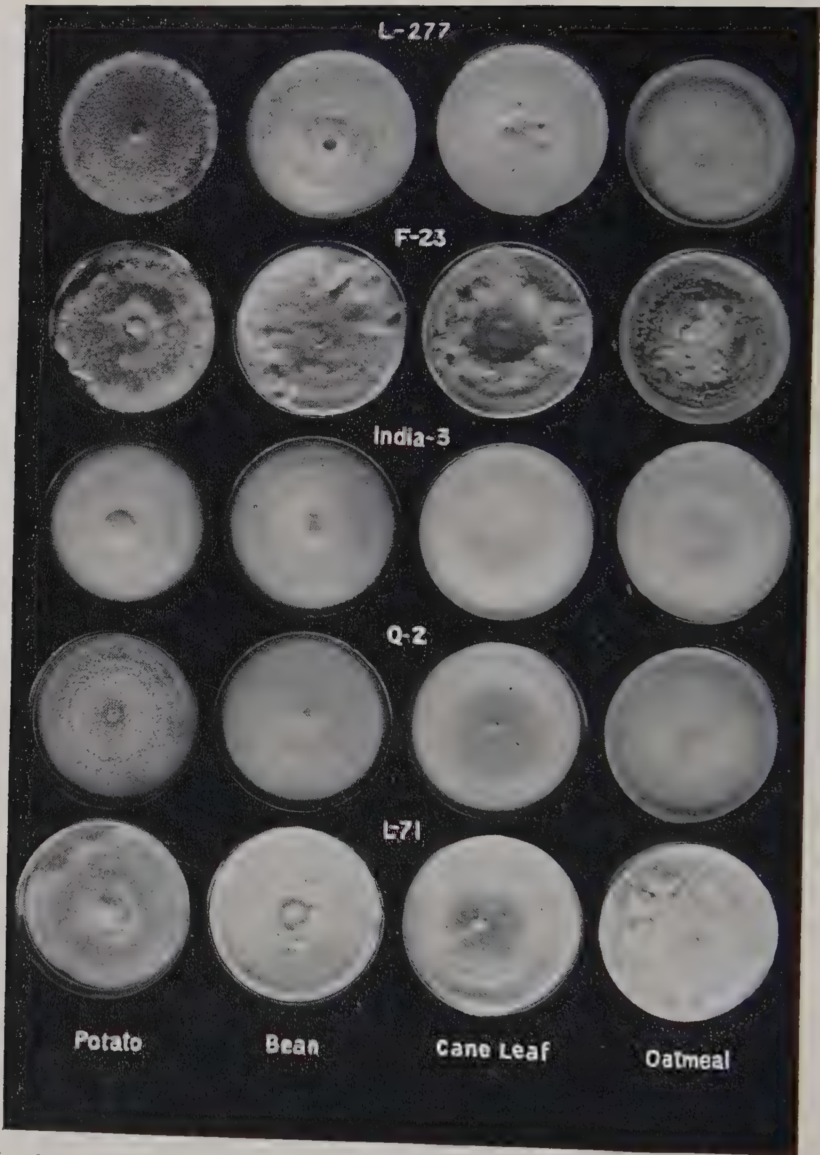
RESPONSE TO CULTURE MEDIA

Colletotrichum falcatum will grow on a variety of plant decoction media, including potato-dextrose, bean-dextrose, oatmeal, and cane-leaf or cane-juice agars. Oatmeal agar was used in most of the cultural work reported in this bulletin because it produced the most luxuriant vegetative development and fructification and more clearly emphasized the differences between the two cultural races. Satisfactory, but less luxuriant growth was obtained on bean, potato, and cane-leaf decoction agars (with dextrose or sucrose added), while scanty and atypical development was obtained on cornmeal, carrot, prune, and Czapek's agars.

Oatmeal agar has the disadvantage, however, of being very opaque and therefore rather unsatisfactory where a clear medium is desired. Various modification of Czapek's medium were tried in an effort to find a synthetic medium on which *Colletotrichum falcatum* would grow satisfactorily. Sources of nitrogen used included peptone, egg albumen, ammonium salts, and nitrates in varying quantities and in different combinations, with sucrose and dextrose as carbon sources,



Comparison of the color and texture of the light and dark cultural races of the red rot fungus on oatmeal agar: *A* and *B*, Light races from Louisiana and Taiwan, respectively; *C* and *D*, dark races from Louisiana and India, respectively; *E*, both races arising from a single piece of cultural tissue from a leaf midrib lesion on Co. 290.



Morphological races of *Colletotrichum falcatum*. Comparison of the colony color and texture of four dark-race isolates of *C. falcatum* (L–277, F–23, India–3, and Q–2) with a typical light-race isolate (L–71) on four culture media. (L=Louisiana; F=Florida; Q=Queensland.)

but growth was uniformly poor on all of them. When a cane-leaf decoction or expressed cane juice was substituted for distilled water or rain water, however, excellent growth was obtained with any of the nitrogen sources mentioned. The medium finally adopted for use, where a clearer medium than oatmeal agar was desired, was prepared as follows: 100 g of finely cut sugarcane leaves (variety C. P. 28/19) were steamed in 1 liter of water for an hour in a double boiler, the decoction was filtered, made up to volume, and the following nutrients added in grams per liter: $NaNO_3$, 5; KH_2PO_4, 1; $MgSO_4$, 1; sucrose, 10; and agar, 10. The cane-leaf decoction was used in preference to cane juice, since leaves were available during a greater portion of the year.

On the decoction alone, growth was very scanty and atypical. The light cultural races fruited after 2 weeks, but the dark ones did not fruit in 30 days. With the addition of sucrose or dextrose the growth was denser than on the decoction alone but less luxuriant than on the medium with nitrogen added also. Both races fruited on the decoction plus sucrose or dextrose but with no nitrogen source added.

DIFFERENTIATION OF CULTURAL RACES

As mentioned in the discussion of the red rot survey, two cultural races of *Colletotrichum falcatum* based on the color and texture of the colony may be distinguished when a large population of the fungus is studied in comparative culture. For convenience, these have been termed light and dark types, respectively. The colony turf of the light type isolates on oatmeal agar is loose, cottony, and floccose, almost white in young cultures, becoming light ashy gray with broad white margins in 7 to 14 days, and dark gray in older cultures (plate 3, *A* and *B*). These isolates usually fruit abundantly over the surface of the colony, frequently forming slimy pink masses of conidia. The turf of the dark types is of a denser, more velvety or felty texture and is dark gray in young cultures, becoming darker with age (plate 3, *C* and *D*.) The white margins are usually lacking in the dark types. They generally fruit sparingly, although conidia are sometimes produced in pink masses around the periphery of the mature colonies, or on the substratum concealed by the superficial aerial mycelium.

These two races are not antagonistic in culture, and evidently not in nature from the fact that isolates of both may be obtained from a single piece of cultured tissue, as shown in plate 3, *E*. Many combinations of representatives of the two races in the same Petri dish have been tried in an effort to produce the perfect stage, but these have not been successful.

INTERMEDIATE TYPES

A few light-type cultures have been obtained that showed a tendency to grade into the color of the dark types, and occasional dark-colored isolates have been encountered that tended toward the looser, more floccose texture and lighter color of the light types. The occurrence of these apparently intermediate forms might suggest a separate classification for them, yet the differences between these isolates as individuals were greater than the differences between them and the more typical isolates of the morphological race to which they were assigned. Whether they represent the normal variation which might

be expected to occur within any morphological group of fungi or are mutants or true genetic hybrids could not be determined, but the fact that similar variations did not arise in culture from any of the monosporous lines studied suggests the latter rather than the former possibility.

CONSTANCY OF CULTURAL CHARACTERS

The study of the morphological characters of *Colletotrichum falcatum* does not appear to be complicated by the occurrence of mutation or other true genetic variation among the biotypes of the fungus. During the course of the present study more than 500 different isolates of the species have been studied at one time or another. Single-spore cultures were made of all of those included in the various morphological and physiological comparisons reported in this bulletin, which involved frequent replating and study of them. In addition, for a few isolates (such as L–31, L–56, L–71, F–1, and G–78), hundreds of mass transfer subcultures have been made over a period of from 5 to 7 years to provide inoculum for the inoculation of the seedlings that are each year tested for red rot in connection with the breeding program. During this time some variation has occurred, such as slight changes in the color of the mycelium, in the degree of fructification, or in the abundance of vegetative growth, but sectoring or other forms of extreme variation have not been observed. Occasionally what appeared to be poorly defined sectors or "patch" variants appeared, but transfers from these produced colonies apparently identical with the original.

The isolates have not only remained stable morphologically, but there has been no significant measurable change in virulence as the result of long continued cultivation on an artificial medium (oatmeal agar). However, no method of measuring virulence has yet been devised that is sufficiently accurate to detect very small differences between races, or slight variations in the virulence of a given isolate that might conceivably occur. The possibility that attenuation of virulence or morphological variations might be induced by subjection to unfavorable environmental conditions has not been investigated.

In addition to this repeated routine examination of subcultures of certain isolates, one experiment was conducted to determine whether sectoring or similar forms of variation might be observed if a larger number of single-spore subcultures were studied at one time. Five light-type and five dark-type isolates were chosen, and from the original monosporous culture of each 25 single-spore subcultures were prepared by the dilution method. Each of these was then carried through two generations by mass transfer on both oatmeal and potato dextrose agars, without observing any significant variations from the original culture.

Some of the isolates studied have shown a tendency to grow less luxuriantly or to become more mycelial after an extended period of artificial cultivation. The latter change was particularly noticeable in the culture L–31, which, when first isolated, was characterized by its early and extremely abundant production of pink masses of conidia over the surface of the culture. After 5 years in culture, however, it no longer fruits with its former abundance. It was observed that successive batches of cultures of a given isolate prepared at different times varied in this respect. Incubation in a dry

atmosphere, for example, tended to suppress fructification, while incubation in a humid atmosphere favored it.

The occurrence of variations in the color of the mycelium and in the degree of fructification in both morphological groups was mentioned in a previous paper (*12*). At that time it was stated that the dark types from the sirup-producing States appeared to be distinguishable from the same cultural races from Louisiana on the basis of these characters. As larger populations of the fungus from both geographic areas were studied, however, such a distinction was not consistently maintained. When isolates of either cultural race are grown on a variety of culture media, sufficient variations occur to suggest that subraces might be differentiated on the basis of response to nutritional variations, but it does not appear at present that such a separation would serve any practical purpose.

These observations on the apparent stability of *Colletotrichum falcatum* in culture do not prove, of course, that new forms of the fungus do not arise in nature. The demonstrated existence of races of the species differing in readily recognized morphological characters as well as measurable differences in virulence (see Comparative Virulence Tests, p. 47) is itself evidence that some influence is or has been operating in nature to produce such variation. The fact that the perfect stage of *C. falcatum* has not been observed does not eliminate the possibility of the origin of new forms through sexual reproduction. Compared with many other fungi, however, such as certain species of *Fusarium* and *Helminthosporium*, *C. falcatum* may be considered a stable species, and it seems logical to assume that the problem of breeding for red rot resistance in sugarcane may be approached with reasonable assurance that the program will not be disturbed by great or sudden changes in the causal organism of the disease.

CONIDIAL CHARACTERS

Since the cultural comparisons had shown that isolates of *Colletotrichum falcatum* fall into two morphological groups on the basis of the color and texture of the mycelial growth in culture, a study of conidial characters was undertaken to determine whether other differences might be found which would serve to further differentiate the two groups. These included size and shape of conidia and the degree of granulation of their contents.

SIZE OF CONIDIA

Conidial measurements were made on 20- to 30-day-old cultures of the various isolates on oatmeal agar, incubated at 25° to 30° C. The measurements were made at different times extending over a period of several years, and each time a new set of isolates was measured, one or more of those previously measured was included in the test for comparative purposes. At least 25, and usually 50, conidia of each isolate were measured in each test, and 42 of the 81 isolates studied were included in at least two tests. A suspension of conidia in 10-percent glycerin in water was used, and all of the conidia encountered in the microscopic field in moving from one side of the slide to the other were measured. Both length and width were determined in the beginning, but when it became apparent that width was less

variable than length, only the latter measurement was made for most of the isolates.

In table 2 are given the range and mean conidial lengths of 54 light-race and 27 dark-race isolates of the fungus, and in figure 4 these are arranged in frequency classes of 1μ grade. The extreme range of length was from 16μ to 48μ; the greatest range for any one isolate was from 20μ to 48μ for G–104; and the smallest was from 20μ to 25μ for L–242. The occurrence of conidia measuring more than 40μ was infrequent, the maximum length for 80 percent of the isolates being

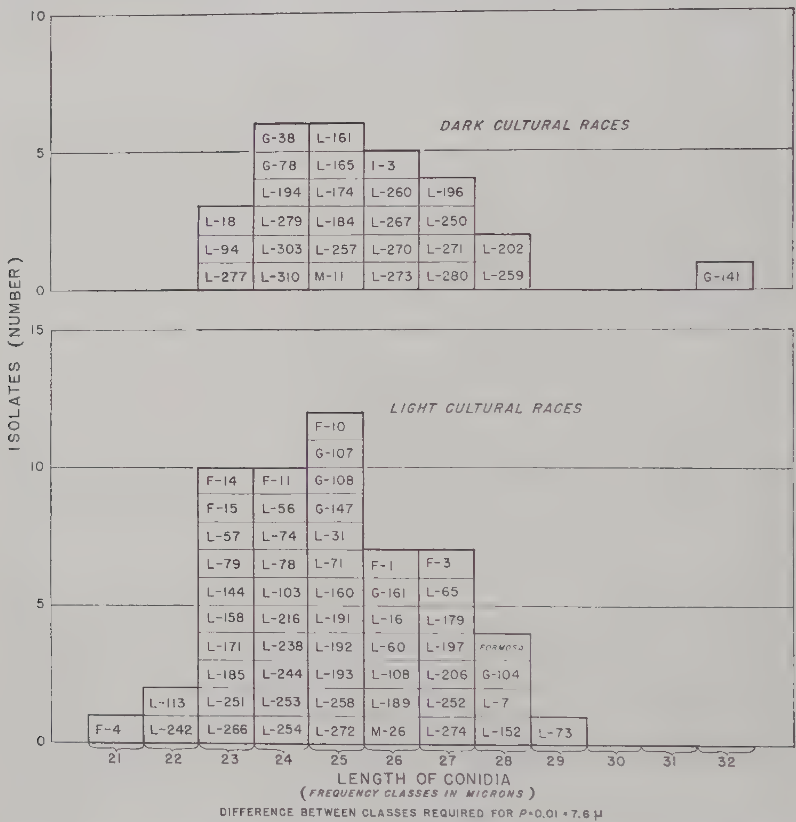


FIGURE 4.—Frequency distribution of 81 isolates of *Colletotrichum falcatum* according to mean length of conidia.

35μ or less. The coefficient of variability of conidial length from a single Petri dish culture was relatively low, varying from 6.3 percent for L–242 to 13.7 percent for G–104.

It is evident from the figures presented that there are no significant differences between the mean lengths for the two cultural groups nor, with few exceptions, between individual isolates. The smallest individual mean length was 21μ for F–4 and the largest was 32μ for G–141, a difference which, while statistically significant, is of little practical importance in view of the many intermediate forms which occur, and the absence of any consistent relationship between cultural

races and spore size. It is likewise obvious that there is no association of isolates of any particular spore length with particular sugarcane varieties. When grouped according to the varieties from which they were obtained, the group means of isolates from any given variety closely approached the general mean for all of the isolates studied.

TABLE 2.—*Length of conidia of 81 isolates of Colletotrichum falcatum from various sources*

[Cultures grown on oatmeal agar at 25–30° C. for 20–30 days]

LIGHT RACES (GROUP A)

Source and isolate No.	Conidia measured		
	Number	Length	
		Extremes	Mean
		μ	μ
From P. O. J. 213 (A-1):			
L-7	50	18–33	28
L-56	133	20–30	24
L-78	63	20–34	24
L-103	27	21–30	24
L-108	133	20–30	26
L-206	27	20–34	27
L-274	53	22–34	27
F-1	350	20–37	26
G-161	75	22–32	26
M-26	75	20–34	26
Subtotal or mean	986	-------	25.8
From P. O. J. 36-M (A-2):			
L-57	106	18–30	23
L-60	103	18–32	26
L-71	53	22–40	25
L-73	51	22–40	29
L-74	50	20–36	24
L-185	50	20–30	23
L-189	26	22–32	26
L-191	50	20–32	25
L-192	26	22–30	25
L-244	26	20–30	24
L-272	50	20–30	25
Subtotal or mean	591	--------	25.0
From Co. 281 (A-3):			
L-31	84	20–30	25
L-65	81	20–32	27
L-79	53	20–32	23
L-144	50	20–28	23
L-179	25	22–32	27
L-238	78	20–30	24
L-242	26	20–25	22
L-253	26	20–30	24
L-254	27	20–36	24
From Co. 281 (A-3)—Con.			
L-266	50	20–30	23
F-3	97	19–33	27
F-4	105	16–31	21
G-147	75	18–32	25
Subtotal or mean	777	--------	24.2
From miscellaneous varieties (A-5):			
L-16	208	20–37	26
L-113	50	20–28	22
L-152	30	20–34	28
L-158	100	20–30	23
L-160	54	20–32	25
L-171	50	20–28	23
L-193	25	22–30	25
L-197	25	24–32	27
L-216	39	20–30	24
L-251	25	20–26	23
L-252	81	22–40	27
L-258	76	20–34	25
G-104	75	20–48	28
G-107	50	20–32	25
G-108	75	20–32	25
Formosa	50	22–32	28
Subtotal or mean	1,013	--------	25.2
From P. O. J. 2714 (A-4):			
F-10	100	20–32	25
F-11	33	18–33	24
F-14	26	20–28	23
F-15	127	18–32	23
Subtotal or mean	286	--------	23.8
Group A, total or weighted mean	3,653	--------	24.9

DARK RACES (GROUP B)

Source and isolate No.	Number	Extremes	Mean
From P. O. J. 36-M (B-1):			
L-184	50	20–30	25
L-271	28	22–38	27
L-273	53	22–40	26
L-303	75	20–30	24
Subtotal or mean	206	--------	25.5
From C. P. 807 (B-2):			
L-18	150	16–33	23
L-277	104	20–30	23
L-279	102	20–30	24
From C. P. 807 (B-2)—Continued.			
L-280	161	20–40	27
G-78	27	20–30	24
Subtotal or mean	544	--------	24.2
From C. P. 28/19 (B-3):			
L-250	25	18–32	27
L-257	109	18–45	25
L-259	53	22–46	28
L-267	156	18–32	26
Subtotal or mean	343	--------	26.5

TABLE 2.—*Length of conidia of 81 isolates of Colletotrichum falcatum from various sources*—Continued

DARK RACES (GROUP B)—Continued

Source and isolate No.	Conidia measured: Number	Length: Extremes	Length: Mean	Source and isolate No.	Conidia measured: Number	Length: Extremes	Length: Mean
		μ	μ			μ	μ
From Cayana (B-4):				From miscellaneous varieties (B-6):			
G-38	130	20-30	24	L-161	51	20-34	25
G-141	40	20-40	32	L-202	28	20-34	28
Subtotal or mean	170	------	28.0	L-260	62	20-31	26
				L-270	27	22-30	26
From Co. 290 (B-5):				L-310	75	20-31	24
L-94	83	20-30	23	I-3	58	22-40	26
L-165	98	18-40	25				
L-174	77	20-32	25	Subtotal or mean	301	--------	25.8
L-194	50	20-28	24				
L-196	25	22-30	27	Group B, total or weighted mean	2,025	--------	25.5
M-11	128	20-30	25				
Subtotal or mean	461	--------	24.8	Weighted general mean, A and B	--------	--------	25.1

The range of length of conidia for the 81 isolates studied is greater than that recorded for the species in the literature, but the weighted general mean of 25.1μ is practically the same as the 25.0μ given by Went (*73*) in his original description of the species. Butler (*27*) gave the range of length as 20μ to 30μ, and Roldan and Tecson (*58*) as 27μ to 34.2μ, but whether these represent the variability of a single isolate or of several was not stated.

EFFECT OF ARTIFICIAL CULTURE ON SIZE OF CONIDIA

The conidial measurements reported in the literature were presumably made of material taken directly from the host. For the accurate comparison desired in the present study, it was necessary that the conidia be produced under the more controlled conditions of artificial culture than would be possible on host material. Artificial cultures also had the advantage of economy of time and space in handling large numbers of isolates. However, in order to determine whether conidia produced by an isolate in culture differed significantly in size from those on the host, measurements from both sources were compared for several isolates.

In July 1933, 10 leaves of Co. 281 were inoculated with each of the following isolates: L-31, L-56, L-71, and L-103. After 30 days, a composite sample of conidia was collected from the 10 leaves inoculated with each isolate, and from 50 to 70 measured. The results are given in table 3.

In comparing these figures with those for the same isolates in table 2, it will be noted that on the host there is a tendency for the lower limits of size to be slightly smaller than in culture. The means, however, were practically identical for three isolates and not significantly different for the other.

TABLE 3.—*Length of conidia of four isolates of Colletotrichum falcatum produced from artificial inoculations on the leaf midribs of Co. 281*

Isolate No.	Conidia measured		
	Number	Length	
		Extremes	Mean
		μ	*μ*
L-31	50	19–33	27
L-56	70	19–30	24
L-71	50	18–29	25
L-103	50	17–31	24

From time to time additional measurements of conidia were made from leaf lesions collected in the field from several varieties, without subsequently making measurements in culture. In all instances, the range of size fell within the limits recorded in table 2. It seems reasonable to conclude that there is no significant difference in size of conidia of *Colletotrichum falcatum* produced on a favorable artificial medium and on the host.

OTHER CONIDIAL CHARACTERS

As the conidia were measured, observations were also made on their shape and the character of their contents. The conidia of some isolates are markedly curved or falcate, while others are nearly straight; the majority are muticate, but some are slightly punctate; the contents of some are densely granular, while others grown under identical conditions are nearly transparent. Differences in the degree of guttation also occur. Granulation and guttation appeared to be influenced by cultural conditions, and for some isolates at least varied from time to time. None of these characters, however, was correlated with length of conidia, nor with the macroscopic characters by which the light and dark cultural races are distinguished.

CHLAMYDOSPORES

Various theories have been advanced as to the function of the chlamydospores, which are produced in such abundance by *Colletotrichum falcatum* both in the host tissues and in artificial culture. These thick-walled, dark-colored cells, which are borne either within or terminally on the hyphae, are capable of survival and germination after detachment from the parent hypha. Went (*73*) first described them and proved their connection with the fungus causing red rot in sugarcane. Butler (*27*) stated that they were better adapted to stand unfavorable environmental conditions than the conidia, and thought they might be the means of perpetuating the disease after their release in the soil from the rotting cane stalks. Lewton-Brain (*46*) observed that in artificial culture they were produced in greater abundance where the fungus came in contact with the substrate, particularly along the walls of the vessel containing the culture. He suggested that this might indicate their nature as hold-fasts by means of which the fungus attached itself to the substratum, but stated there

was no evidence to support this view. Butler and Hafiz Khan (*30, p. 170*) state that it is probable that "they serve a double purpose of close adhesion to the surface of the host plant and of accumulation of enzymic energy to secure penetration of its walls." They found that the infection hyphae in leaf infections (from artificial inoculations) usually arose from a chlamydospore, or appressorium.

In the present study the author has observed that in artificial culture the dark cultural races produce chlamydospores in much greater abundance than the light races. This is particularly true, as noted by Lewton-Brain (*46*), at the point of contact of the culture medium with the glass of the test tube or culture dish. The mycelium of the dark races at this point may consist largely of masses of chlamydospores. The mention by Went, Lewton-Brain, and Butler and Hafiz Khan of the abundant production of chlamydospores in their cultures suggests to the author that they may have been working with the dark cultural races.

Butler and Hafiz Khan's (*30*) description of the chlamydospores as appressoria seems to offer a logical explanation of the natural function of these organs, and if sugarcane leaves and stalks in nature were not subject to attack by insects which injure the epidermis, thus permitting *Colletotrichum falcatum* to gain easy entrance into the host tissues, the production of chlamydospores as organs of infection, might be essential to the parasitic life of the fungus. They found that when leaves were artificially inoculated without wounding, the penetrating hypha arose from an appressorium and not directly from a conidium. Whether the appressorium was produced prior to invasion of the wounded leaves was not stated. The author has found, in artificially inoculating sugarcane stalks with red rot, where a small hole is punched through the epidermis and a culture of the fungus inserted into the tissues (simulating infection through borer holes), that the fungus hyphae penetrate the adjacent cell walls without the production of appressoria. Whether the infection hyphae initiating infections in borer tunnels in nature arise directly from a conidium or a chlamydospore has not been determined, but it appears improbable that the chlamydospore functioning as an appressorium is essential under such conditions.

TEMPERATURE RELATIONS

Studies of the temperature range of *Colletotrichum falcatum* in culture were made at Arlington Experiment Farm, Arlington, Va., in 1932, where a series of constant temperature chambers was made available through the courtesy of the Division of Fruit and Vegetable Crops and Diseases.

Ten Petri plates of oatmeal agar were inoculated with each isolate used in the tests by touching a mass of conidia with a needle point and then transferring to the center of the plate. The plates were wrapped in paper, placed in the chambers, and the diameter of the colonies measured after 3, 4, 6, and 8 days' incubation. The chambers were adjusted to maintain the following temperatures: 0°, 5°, 10°, 15°, 17.5°, 20°, 22.5°, 25°, 27.5°, 30°, 32.5°, 35°, and 37.5° C. In some of them the temperature fluctuated about 1°. Since no growth had occurred below 10° after 8 days, the plates at 10° were continued for 2 weeks and those at 0° and 5° for 1 month. Humidity was maintained by placing a tray of water on the floor of each chamber, and a

small fan in each prevented layering of air. The growth measurements for two typical isolates are presented in table 4.

It will be seen from the table that the minimum temperature at which *Colletotrichum falcatum* will grow is approximately 10° C. and the optimum, 30° to 32.5° C. Sartoris (*60*) obtained slight growth at 3° C. after first incubating for 36 hours at 20° C. and then transferring to the lower temperature.

The upper limit of growth is slightly above 37.5° C. At this temperature scant atypical development occurred, and in subsequent tests no growth was obtained at 40° C.

Edgerton and Moreland (*36*) found that the organism would grow at temperatures ranging from 13° to 37.5° C., with the optimum being "somewhere in the neighborhood of 27° C." Tims and Edgerton (*67*) reported that growth was very slow at 10°, rapid at 27° to 34°, and slow at 37° C.

TABLE 4.—*Growth of two isolates of Colletotrichum falcatum at various temperatures on oatmeal agar*

[Means of 10 plates]

Temperature (° C.)	Diameter of colonies							
	Isolate No. L-7				Isolate No. F-1			
	3 days	4 days	6 days	8 days	3 days	4 days	6 days	8 days
	Cm	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	.0	.0	.0	[1].0	.0	.0	.0	[1].0
10	.0	.0	G [2]	G	.0	.0	.0	.3
15	G	G	1.9	3.3	G	G	2.3	3.6
17.5	.7	1.9	3.8	5.1	.9	2.2	4.3	5.5
20	1.5	2.6	3.9	5.1	1.9	3.0	4.9	6.4
22.5	2.2	4.0	4.7	8.2	2.4	3.9	5.8	8.3
25	3.4	5.2	6.8	([3])	3.2	5.3	8.2	--------
27.5	2.6	6.0	8.8	--------	3.4	5.9	8.9	--------
30	3.0	6.0	--------	--------	4.1	6.2	--------	--------
32.5	3.3	4.7	7.3	--------	4.0	5.8	8.5	--------
35	1.8	2.5	3.6	4.9	1.7	2.8	3.4	4.6
37.5	.5	1.5	2.5	3.0	G	.6	.9	1.5

[1] No growth occurred at this temperature after 30 days.
[2] G indicates germination only.
[3] Indicates that the growth had filled the plates and could not be measured.

Subsequent tests have been made with representative populations of both cultural races in constant temperature chambers at Houma, La., the results of which have been in accordance with those in table 4. All isolates studied were found to have the same critical temperatures, indicating that temperature response cannot be used as a basis for differentiating races.

RATE OF GROWTH IN CULTURE

Growth-rate measurements in artificial culture were made of both light and dark races in an effort to determine whether this function might be correlated with the morphological differences described and serve as a further means of differentiating the cultural races.

Five Petri plates of cane-leaf decoction agar were inoculated with each isolate used in the tests and incubated for 6 days at 30° C., at

the end of which time the diameter of the colonies was measured. An effort was made to transfer a uniform amount of inoculum to each plate, but in order to eliminate error from this source, the limits of growth in each plate were indicated at the end of 2 days' incubation and this was subtracted from the final reading in recording the results. The results of three representative growth measurement tests appear in table 5.

TABLE 5.—*Mean radial growth of 17 isolates of Colletotrichum falcatum after 4 days' incubation on oatmeal agar at 30° C.*

Type and isolate No.	Test 2	Test 3	Test 5	Mean	Variance (1)
	Cm	*Cm*	*Cm*	*Cm*	
Light:					
L-31	5.9	6.2	6.1	6.1	0.025
L-73	6.1	6.0	6.0	6.0	.005
L-94		6.3	6.1	6.2	.020
L-108		6.0	5.8	5.9	.020
L-171	5.8	6.0	6.1	6.0	.025
L-258	5.9		6.0	6.0	.010
F-11		6.2	6.2	6.2	.000
F-14		6.3	6.1	6.2	.020
General mean				6.1	
Dark:					
L-173	5.8	6.4		6.1	.180
L-184	5.8		5.8	5.8	.000
L-194	5.7		6.3	6.0	.180
L-202	5.8	6.4		6.1	.000
L-250	5.8		5.8	5.8	.000
L-257		6.0	5.5	5.8	.130
G-78		5.9	6.5	6.2	.180
G-100	5.7	6.0	6.5	6.1	.165
M-14		6.5	6.5	6.5	.000
General mean				6.0	
Weighted mean variance					.052

[1] Standard error of single isolate mean ($\sqrt{0.052}=0.228$); standard error of difference between single isolate means ($\sqrt{2}\times0.228)=0.322$; difference between individual means required for significance (odds 100:1) $=0.322\times3=0.97$ cm or 1.0 cm.

The average mean radial growth of eight light types, 6.1 cm, was practically the same as that of nine dark types, 6.0 cm. The means ranged from 5.8 cm for several dark types to 6.5 cm for M-14, also a dark type. These differences between extremes were too small to be significant, and it was concluded that rate of growth in culture (at the optimum temperature for the organism) is not correlated with the morphological differences distinguishing the cultural races.

INFLUENCE OF pH OF CULTURE MEDIUM ON RATE OF GROWTH

For the study of the influence of varying pH on the growth of *Colletotrichum falcatum* in culture, a large batch of oatmeal agar was prepared and aliquots were adjusted colorimetrically, representing a range in pH from 4.0 to 9.0 at intervals of from 0.5 to 1.0. A set of standards was set up with Clark and Lubs indicators according to the method described by Medalia (*49*). The reaction of the medium as prepared was pH 5.8 to 6.0, and appropriate quantities of N/10 HCl and NaOH were added to give the desired pH range.

The acid and alkali were added to the medium after sterilization and before pouring. Five plates were poured from each aliquot for each isolate studied and the inoculations made by touching the center of the plate with a needle, which had been dipped into a pink mass of

conidia. The plates were incubated at 25° C., and the diameter of the colonies measured at the end of 5 and 6 days. The results of the measurements for two light-type isolates, F–1 and L–7, are presented in table 6.

TABLE 6.—*Influence of varying pH of culture medium (oatmeal agar) on growth of Colletotrichum falcatum*

[Incubated 6 days at 25° C.; means of five plates of each isolate]

Isolate No.	Average diameter of colonies at pH—							
	4.0	4.5	5.0	5.5	6.0	7.0	8.0	9.0
	Cm	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*
F–1	1.8	6.7	8.7	9.0	8.8	8.6	8.6	7.5
L–7	1.5	6.4	8.6	8.9	8.8	8.5	8.4	7.3

Maximum growth occurred between pH 5.0 and 6.0, with the optimum apparently near pH 5.5. It is of interest to note that this is approximately the reaction of freshly expressed juice of some sugarcane varieties in Louisiana (*65*).[9]

At pH 4.0 the colonies were not typical, the growth consisting of a dense blackish-gray film over the surface of the medium. There was no fruiting. At 4.5 growth was more nearly typical, but the colony was still more dense than normal and the reverse greenish black. There was slight fructification. At pH 5.0, 5.5, and 6.0 the growth was normal in every respect. At pH 7.0 to 9.0 it was similar to that at 4.5.

REVISED DESCRIPTION OF COLLETOTRICHUM FALCATUM

Some modification of Went's original description (*73*) of *Colletotrichum falcatum* is necessary to cover the range of variation shown by the hundreds of isolates included in the present study. Since he and later workers have given little information on the cultural characters of the organism, the following description has been prepared:

Colony on oatmeal agar consists of abundant aerial mycelium, broadly spreading, sometimes zonate, densely woven into a compact, velvety turf in some isolates (dark races), or a cottony, floccose one in others (light races). (Plate 3.) Color ranges from almost white through light ashy gray to dark gray, growing darker with age (pale olive gray to pearl gray); no color or pigmentation in reverse or in the medium. Hyphae densely interwoven, anastamosing in definite ropes. Conidia formed in pink masses in stromata, or singly on scattered conidiophores over the aerial hyphae. Conidia one-celled, mostly falcate, but some straight; muticate or slightly punctate; transparent to densely granular; frequently guttulate; 16μ to 48μ long by 4μ to 8μ wide, averaging 25μ by 6μ. Terminal and intercalary thick-walled, greenish-black chlamydospores present, generally more abundant in the dark races. Empty, black pseudopycnidia sometimes produced.

Habitat.—On leaf midribs and in stems of species of *Saccharum*; on leaf midribs of *Sorghum vulgare* Pers., *S. halepense* (L.) Pers., and *Erianthus giganteus* (Walt.) Muhl.

[9] The pH of freshly expressed juice of mature stalks of P. O. J. 213 (rind removed) was 5.47 and of Co. 281, 5.34. Electrometic determinations by S. J. Breaux.

Growth on cane-leaf, green-bean, and potato decoction agars with sucrose or dextrose is similar to that described, but less luxuriant, as will be noted from plate 4.

The above description of *Colletotrichum falcatum* agrees essentially with those of other workers, differing in that it covers a wider range of variation of characters. Went (*73*), for instance, gave the conidial size as 25μ by 4μ; Butler (*27*) as 20μ to 30μ by 5μ to 7μ; and Roldan and Tecson (*58*) as 27μ to 34.2μ by 4.3μ to 6.8μ, all of which come within the range of size found by the author. Their descriptions were of material from the living host, however, and not from artificial cultures.

COMPARISON OF AMERICAN AND FOREIGN ISOLATES

At the Fourth Congress of the International Society of Sugar Cane Technologists, held at San Juan, Puerto Rico, in 1932, it was suggested by the Pathology Section that sugarcane pathologists throughout the world cooperate in the collection of strains of pathogens by workers in responsible institutions specializing in the study of them. Accordingly, cultures of *Colletotrichum falcatum* were requested from several countries where red rot occurs. Isolates of the fungus were received through the courtesy of A. F. Bell, Bureau of Sugar Experiment Stations, Brisbane, Queensland, Australia; B. B. Mundkur, Imperial Institute for Agricultural Research, Pusa, India; and Takashi Matsumoto, Taihoku Imperial University, Taihoku, Taiwan. These were compared in culture with the American isolates.

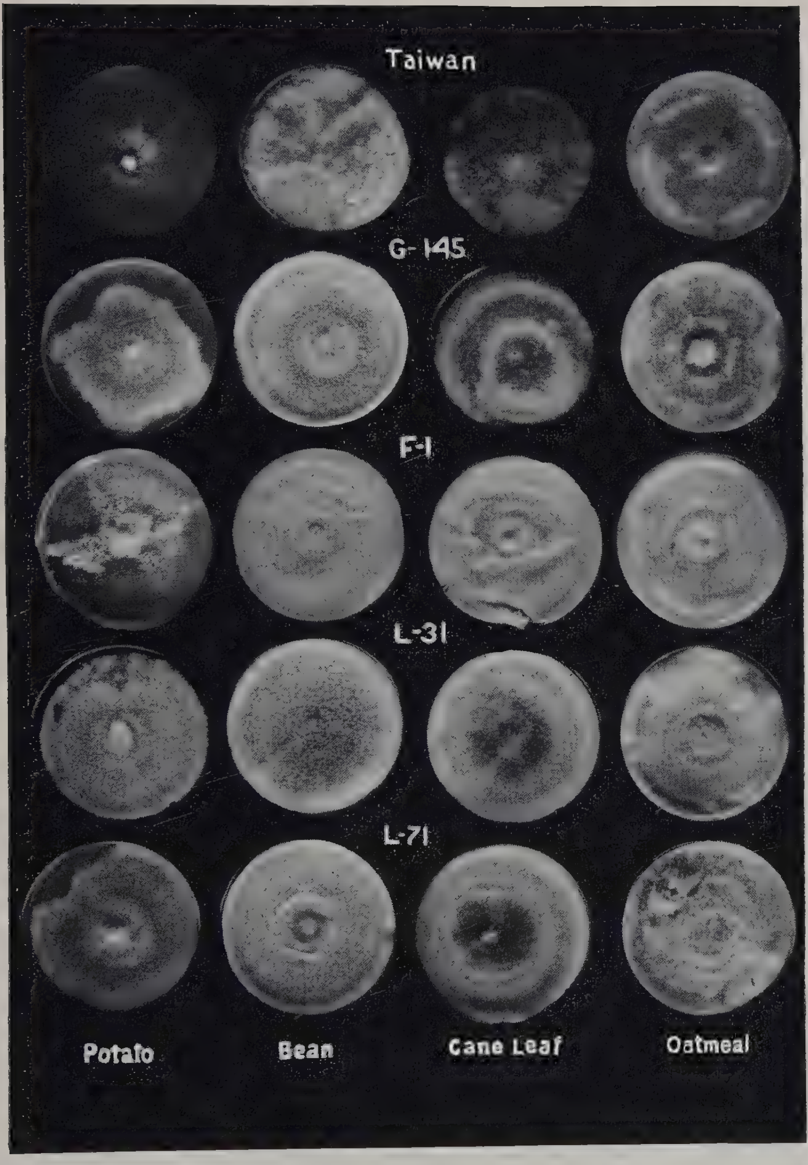
The Indian and Australian cultures were morphologically similar to the American dark-race isolates, while the Formosan (Taiwan) culture closely resembled the American light races on oatmeal and on bean agar (plates 3, 4, and 5). Conidial measurements of the foreign isolates fell within the range of variation found for the American forms.

It is evident from this comparison that both morphological groups of *Colletotrichum falcatum* occur in the Eastern Hemisphere, a fact of considerable importance in view of the results of the survey of the flora in the United States, and of the comparative virulence tests (see p. 47) showing differences in virulence between the two groups. If the light races were known only from the United States and the dark races only from other countries, it might have been presumed that the more virulent light races were of American origin. The existence of both morphological races in the Eastern Hemisphere, however, even though little is known regarding their distribution, indicates that both probably originated there and were introduced into the United States in cuttings of sugarcane of which numerous varieties were imported from these regions prior to the inauguration of the Federal quarantine.

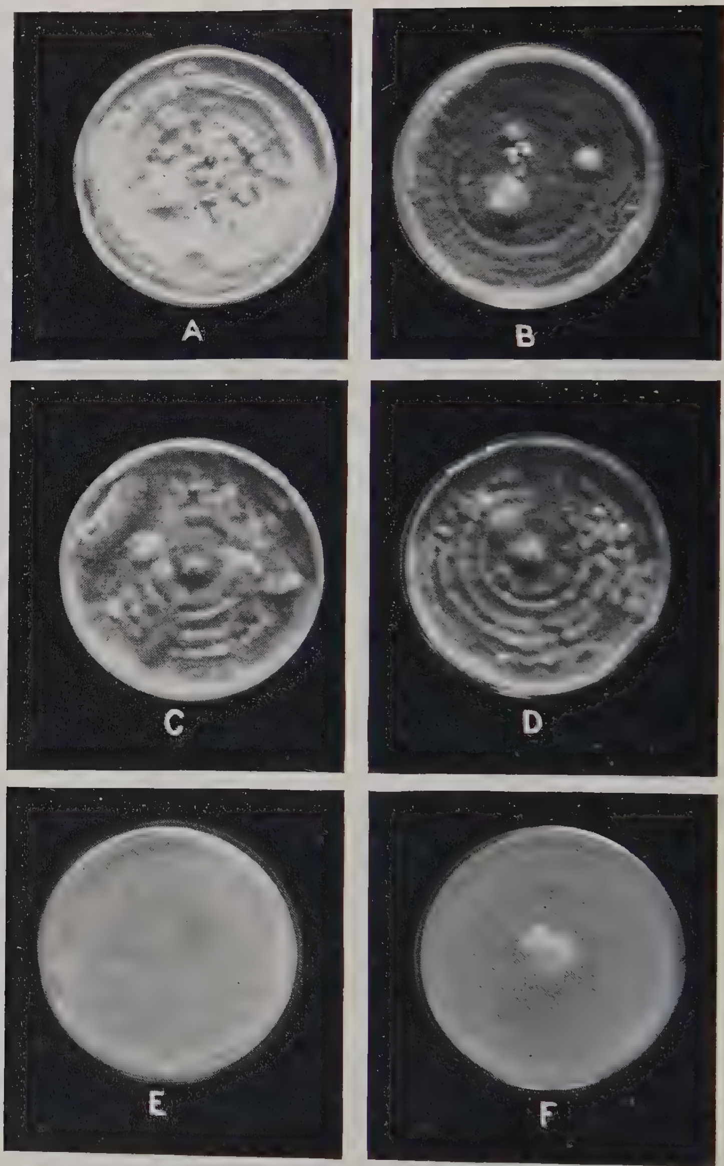
COMPARISON WITH OTHER GRASS SPECIES OF COLLETOTRICHUM

According to Edgerton (*33, pp. 7–8*):

> The fungus which causes the Red Rot of sugarcane is very closely related to two other fungi which we have in this country. These are *Colletotrichum lineola*, found commonly on Johnson grass and broom corn in the southern states, and *Colletotrichum cereale*, found on wheat and other grasses in the northern states. These three fungi are so much alike it that is impossible to distinguish them with the microscope or in pure cultures.



Cultural characters of light races. Range of variation in type of growth on four culture media shown by five light-race isolates of *Colletotrichum falcatum*. (G=Georgia; F=Florida; L=Louisiana.)



Representative dark-race isolates of *Colletotrichum falcatum* from various sources in comparison with the cereal leaf fungus, *C. graminicolum*, on oatmeal agar: *A*, From sorgo; *B*, sugarcane; *C*, plumegrass; *D* and *E*, Johnson grass, compared with *F*, *C. graminicolum*. *D* and *E* represent different isolates of the fungus from Johnson grass and illustrate the variation in cultural characters which may occur among the dark-race isolates. *D* closely resembles the sorgo, sugarcane, and plumegrass isolates, while *E* is more similar to *C. graminicolum*. *A* and *E* represent the intermediate forms described on page 25.

In inoculation experiments, he failed to obtain satisfactory infection of sugarcane stalks with the two grass species. *Colletotrichum lineola* Cda. could be found within the vascular bundles of the inoculated cane, but it did not produce the typical symptoms of red rot. He expressed doubt as to whether the three were valid species, or merely strains of one.

Butler and Hafiz Khan (*30, p. 169*) stated:

> The only other *Colletotrichum* resembling *C. falcatum* found widely distributed in India, is *C. Lineola* Corda, which attacks the leaves of jowar (*Andropogon Sorghum*) frequently. Morphologically the two species are closely allied, but the jowar fungus does not attack cane leaves.

They concluded that *C. falcatum* appeared to be confined to sugarcane.

Wilson (*75*) grouped the several species of *Colletotrichum* described from grasses and a number that had been assigned to related genera, into the single new combination, *C. graminicolum* Ces. Wils., including *C. lineola* Cda. and *C. cereale* Manns in the synonymy for that species. This classification now appears to be generally accepted. Böning and Wällner (*24*) added further to the synonymy, but noted that *C. falcatum* should be excluded because of its sickle-shaped conidia and the occasional production of two in chainlike form.

In the present work many isolates of *Colletotrichum* from leaf midrib lesions have been made from Johnson grass (*Sorghum halepense*), sorgo (*Sorghum vulgare*), and plumegrass (*Erianthus giganteus*) in Louisiana. The leaf lesions on these hosts were similar in appearance to those produced by *C. falcatum* on sugarcane leaves, and the isolates obtained, which belonged to both the dark and light cultural races, were indistinguishable in culture from those from sugarcane (plate 6). When inoculated into sugarcane stalks, the isolates from the other hosts produced typical red rot symptoms, and they were reisolated from the affected tissues. A study of conidial and other morphological characters showed definitely that they should be classified as *C. falcatum*.

Two cultures of *Colletotrichum graminicolum* were studied by the author, one supplied by J. C. Gilman, of the Iowa State College; and the other received from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands (G. Bd.). Information as to the host from which obtained was not available for either culture. These were compared in culture and in laboratory inoculation experiments with isolates of *C. falcatum* from sugarcane and other grasses.

On oatmeal agar the Iowa culture of *Colletotrichum graminicolum* showed little resemblance to *C. falcatum*, the colony of the former consisting of scant, grayish-green aerial mycelium with abundant production of empty, black pseudopycnidia, in contrast with the luxuriant turf of *C. falcatum* and the absence of, or rare production of, pseudopycnidia. The Netherlands (G. Bd.) culture of *C. graminicolum*, however, closely resembled some of the dark-race isolates of *C. falcatum* (plate 6, *F*). (Also compare plate 6, *F*, with plate 4, India-3 and Q-2.) The dark gray color of the turf of the *C. graminicolum* was identical with that of many isolates of *C. falcatum*, but the texture of the former was more threadlike than the latter and did not produce the abundant aerial growth characteristic of *C. falcatum*. However, it could not be distinguished readily from many cultures of

the latter species, particularly some that grow less luxuriantly after long-continued artificial culture.

Microscopically, the conidia of *Colletotrichum graminicolum* were predominantly straight rather than sickle-shaped, as described by Böning and Wällner (*24*), were shorter (15μ to 23μ long) than described by these authors for *C. graminicolum*, and shorter than the majority of the isolates of *C. falcatum* studied. Böning and Wällner stated, however, that the culture of *C. graminicolum* at Baarn differed in some respects from their cultures of the species obtained from corn.

In inoculation experiments with *Colletotrichum graminicolum* there was no spread of the fungus in the tissues of red rot-susceptible varieties of sugarcane after 4 weeks' incubation at a favorable temperature.

It is evident from the literature and from the present study that at least some strains of *Colletotrichum falcatum* and *C. graminicolum* are morphologically similar, although the author found that other strains bear little morphological resemblance. It is likewise apparent that considerable diversity of morphological characters exists among the various fungi that have been classified as *C. graminicolum*, a species that has not yet been adequately studied to determine the range of variation which may occur among the fungi properly referred to it. Aside from certain morphological differences between *C. falcatum* and *C. graminicolum*, the fact that the latter does not infect sugarcane provides ample evidence that the sugarcane and cereal fungi are distinct. Since the cultures of *Colletotrichum* obtained by the author from the leaves of Johnson grass, plumegrass, and sorgo in the sugarcane district of Louisiana were morphologically indistinguishable from isolates of *C. falcatum* obtained from sugarcane, and produced typical symptoms of red rot when inoculated into sugarcane leaves and stalks, it is concluded that the fungus commonly attacking the leaves of the grasses mentioned in southern Louisiana is *C. falcatum*. While *C. graminicolum* may also be present there, it has not been isolated in the present studies, and would appear to be much less common than the former species.

No attempt was made to determine the range of hosts of *Colletotrichum falcatum* beyond those on which it was found naturally occurring, and no claim is made that the hosts mentioned are the only ones on which it occurs. From the practical standpoint of persistence and spread of the red rot fungus in fields of sugarcane, its other wild and cultivated hosts appear to be of little significance in view of its universal occurrence on sugarcane itself and its ability to develop actively on this crop throughout the year without the intervention of a secondary host.

LIFE HISTORY OF COLLETOTRICHUM FALCATUM IN RELATION TO OCCURRENCE AND SPREAD OF THE DISEASE

Although red rot may infect almost any part of the sugarcane plant, its importance is limited largely to its occurrence on the leaf midribs, the interior of the stalks, and the rhizomes of the ratoons. While infection of the roots may occur (*5*, *27*), red rot is not important as a root disease.

ON THE LEAVES

A consideration of the life history of the fungus may conveniently begin with the lesions produced on the leaf midrib. These lesions are usually abundant in the field during the late summer and fall months, particularly on the commercial varieties Co. 281, Co. 290, C. P. 28/11, C. P. 28/19, and C. P. 29/116. On these varieties the spots frequently extend almost the entire length of the midrib (plate 2). Other varieties, such as P. O. J. 213, C. P. 807, and Louisiana Purple, the stalk tissues of which are very susceptible to red rot, generally show little leaf infection. However, such an inverse correlation does not exist with many varieties.

In Louisiana, the first leaf infections are usually noted during May or June. While the majority of these lesions probably originate in punctures made by insects, Butler and Hafiz Khan (*30*) found, and the writer has obtained confirmatory evidence, that infection will take place through the apparently uninjured epidermis. Fructification begins in 10 to 14 days following inoculation, and as the lesions extend longitudinally along the midrib, the fruiting area likewise increases so that old lesions are usually covered with black masses of ascervuli.

The disease on the leaves probably has little effect on the growth of the plant, although it sometimes causes premature drying of the lower leaves of some varieties (principally unreleased seedlings). The importance of the leaf lesions lies in the fact that they provide the principal source of inoculum for stalk infections and for disseminating the disease during the growing season.

This multiplication of the fungus on the leaves may be of considerable importance when a variety very susceptible to leaf infection is introduced into a section where another variety grown there may be very susceptible to the disease in the stalk, but normally has little leaf infection. This appeared to have occurred on at least one plantation near Bunkie, La., in 1935–36, when the first important reduction in germination occurred in the very susceptible P. O. J. 213, which had been grown there for a decade without such injury. The evidence indicated that this injury was correlated with the expansion in acreage on this plantation in 1935 of the recently introduced C. P. 28/19, plantings of which were made adjacent to those of P. O. J. 213. It was noted in the fall of 1935 that the P. O. J. 213 adjacent to the C. P. 28/19, nearly every leaf midrib of which had long red rot lesions, showed a greater incidence of red rot in the stalks than had ever been observed in this variety in that section of the State. Isolations from the leaves of the C. P. 28/19 and the stalks of the P. O. J. 213 showed that the forms of *Colletotrichum falcatum* from both varieties were of the light-colored morphological race.

Fall plantings of P. O. J. 213 were made on the plantation with seed cane from this field adjacent to the C. P. 28/19. In the spring, a gappy stand resulted in this variety, which in this section of the State had largely escaped the severe losses experienced during the earlier epidemics affecting it in other parts of the sugar district. Examination of the seed cuttings showed that the reduction in germination was due to red rot, and light race isolates were obtained from the rotted cuttings. This instance illustrates the possibility of building up strain populations of *Colletotrichum falcatum* on the leaves of one variety,

which may cause a destructive increase of the disease in the stalks of another variety on which such an increase would not have occurred because of its resistance to leaf infection.

Unless the population of *Colletotrichum falcatum* so multiplied is virulent toward the susceptible variety exposed to it, there may be little influence on the incidence of the disease in the susceptible variety. This is illustrated in Georgia where, as shown in the surveys, the production of enormous quantities of inoculum of the dark morphological race of *C. falcatum* on the leaves of Cayana, a high percentage of the leaves of which always have an abundance of lesions, has not resulted in an increase in the disease in the stalks of P. O. J. 213. Conversely, Cayana grown in Louisiana seldom has more than a trace of leaf infection, even though it be exposed to heavy inoculum from adjacent varieties, such as Co. 281 and C. P. 28/19, on which both light and dark races are present.

IN THE STALKS

STANDING CANE

The fungus may enter the sugarcane stalk through various channels. Went (*73*) concluded that natural infection occurred chiefly through the holes made by boring insects. Butler (*27*) and Butler and Hafiz Khan (*30*), however, found little or no natural infection through borer holes in India. Lewton-Brain (*46*), Edgerton (*33*), and South and Dunlop (*1*) agreed with Went that borer holes were the principal means of natural infection of the stalk. Raciborski (*53*) described the spread of the fungus from the seed piece to the growing stalk, which was confirmed in India (*27*, *30*), but not in Louisiana (*33*) or the West Indies (*1*). The root primordia (*30*), leaf scars (*41*, *73*), mechanical wounds, and growth cracks [10] (*30*) have also been described as points of entrance. According to Butler and Hafiz Khan (*30*) old leaf scars are not readily penetrated, and since the leaf scars are normally not exposed until the leaf has completely withered, they were not considered an important point of entrance. Natural infection through the leaf scars has not been reported in the United States.

In Louisiana and southern Florida infection of the growing stalk occurs principally through the tunnels of the moth borer (*Diatraea saccharalis* F.) (plate 7, *C*) and, in some varieties, through the root primordia (plate 7, *A* and *B*). The latter type of infection is limited largely to certain very susceptible varieties, such as P. O. J. 213 in Louisiana and P. O. J. 2714 in southern Florida. Underground portions of the stalk may become infected through injuries made by the sugarcane weevils (*Anacentrus* sp.). Infection from this source seldom extends into the above-ground internodes, but tonnage may be reduced through retardation in the growth of the cane. The principal effect of infection of the rhizome is that it may lead to stubble deterioration.

In most of the sirup-producing areas of the South, there is little development of red rot in the stalks of growing cane due to the absence or infrequent occurrence of the moth borer. Infection through the root primordia and subsequent spread in the stalks usually do not

[10] LOUISIANA STATE UNIVERSITY, DEPARTMENT OF PLANT PATHOLOGY. REPORT OF THE CONTACT COMMITTEE. July 12, 1935. [Mimeographed.]

Typical spring condition of fall-planted seed cuttings: *A*, Co. 281, resistant; *B*, P. O. J. 213, very susceptible, showing infection originating through uninjured nodes; *C*, completely rotted stalk of P. O. J. 213 invaded through borer tunnels.

occur early enough in the season to lower seriously the quality of the cane for sirup by the time it is harvested.

As above mentioned, midrib lesions serve as the principal source of inoculum for stalk infections, the frequent winds and dashing summer rains providing an excellent medium for disseminating the myriads of conidia by splashing and blowing them to the stalks or to other leaves, or by trickling over the leaf lesions and down to the stalk, where drops of water containing conidia may be held for several hours by the cuplike ligule. The fungus continues to develop in the tissues of both the midrib and the stalk until the cane is cut in the fall for seed or for the mill. In the stalks, longitudinal spread through the vascular bundles is more rapid than lateral spread through the parenchyma, as Edgerton (*33*) has pointed out. The cane that goes to the mill plays no further part in the life history of the disease, except through the possibility that the fungus may survive the milling process (which experiments have shown to be possible), and subsequently spread to growing plants from the bagasse.[11]

SEED CANE

Mode of Infection

In Louisiana, where a large percentage of the crop is planted in the fall, seed cane is commonly laid down with many of the leaves and sheaths adhering to the stalks. This means that an abundant supply of *Colletotrichum falcatum* goes into the soil with the seed cuttings, in addition to that which already may have established itself within the stalk and in the soil. Further infection may then take place from this inoculum through borer holes or through the nodes. Some very susceptible varieties, such as P. O. J. 213, in Louisiana show a high degree of infection from the latter source (plate 7, *C*). Other varieties, such as C. P. 807, the internal tissues of semidormant cuttings of which are highly susceptible once the pathogen has gained entrance, are resistant to nodal infection under usual field conditions. During the epidemics of 1934–35 and 1935–36 on this variety, however, nodal infection occurred commonly, and was in fact responsible for much of the injury which occurred (*11*).

Inoculation experiments have shown that infection through the cut ends of seed pieces may also occur. Cuttings of P. O. J. 213, obtained from northern Louisiana where the moth borer is rare and where red rot has not been recorded in standing cane of the variety, were inoculated by smearing an agar culture of *Colletotrichum falcatum* over the cut ends. Borer-free cuttings of C. P. 807, which usually shows little red rot infection as standing cane, were selected at the Houma station and inoculated in the same manner. Both varieties were planted in the field in October and dug up for examination at intervals during the winter. A small percentage of the cuttings of

[11] *Colletotrichum falcatum* was recovered in culture on two occasions (December 1931 and January 1932) from bagasse of P. O. J. 213 collected from the last roller (12-roll Fulton mill) of the Southdown Plantation factory, Houma, La. The fungus was recovered immediately after collecting the bagasse by culturing the pieces of the bagasse on acidified oatmeal agar, and for 3 months thereafter from a pile stored under shelter outdoors. After April it could not be recovered in culture by direct plating of pieces of the bagasse, possibly because of overgrowth of the plates by *Trichoderma* and other rapid-growing saprophytes. Inoculations into stalks of P. O. J. 213 were then resorted to, using pieces of the bagasse as inoculum. Viability of *C. falcatum* was proved by the production of red rot in the inoculated cane up until August 1932, after which the inoculations were negative. This proved that the red rot fungus may survive in bagasse for at least 7 months after coming from the mill.

both showed penetration of the fungus from the cut ends through the nodes and into the adjacent internodes. In subsequent experiments in the laboratory, in which borer-free and red rot-free cuttings of P. O. J. 213 and Co. 281 were inoculated as above and placed in moist chambers, the fungus spread through the nodes from the cut ends and invaded the internodes.

While these experiments proved that red rot infection of seed cuttings may occur through the cut ends, extensive examination of seed cane of several commercial varieties in plantation fields over a period of several years indicates that this source of infection is of relatively little practical significance, the other modes of infection described being of far greater importance. The ends of seed cuttings soon become overgrown with yeasts and other organisms, as pointed out by Edgerton and Moreland (*36*), or they ferment, both of which serve to prevent the growth of the red rot fungus.

Spread Within the Stalk

The rate of spread of red rot within the seed cutting after planting depends to a considerable extent on the degree of susceptibility of the sugarcane variety. It is greatly influenced, however, by soil temperature and moisture, both of which also have an important bearing on the growth of the cane plant itself. The relation of these and other environmental conditions to the development of the disease will be discussed later.

Under favorable conditions, the red rot fungus spreads rapidly through both the vascular bundles and the parenchyma, and all of the internal tissues of the stalk may be invaded within 2 or 3 months after planting (see Pathological Anatomy, p. 85). Dense wefts of mycelium develop in the pith cavities, and in the advanced stages of rotting fructification may occur in these cavities and on the exterior of the stalk. In the spring, when the cultivation operations begin, pieces of seed cuttings are frequently left exposed along the rows, which, together with the trash from the previous season's growth, serve as sources of inoculum for the leaves of the new crop. Stubble rhizomes also serve the same purpose.

SIRUP-PRODUCING SECTIONS

In the sirup-producing areas, cane is not planted in the fall but stored for spring planting in piles commonly termed "banks" or "beds." The bedding is done by making low piles of mature stalks with tops and leaves adhering, so placed that the tops of some plants cover the stalks of others. The beds, which are commonly about 3 feet wide and 2 to 3 feet in height, are completed by covering the cane with several inches of soil. In these beds the seed cane may become infected by red rot through the root buds during the winter (fig. 5), and to some extent through the cut ends, the conidia on the adhering leaves providing an abundant source of inoculum. Because of this, a variety like Cayana, the leaves of which are very susceptible to the disease, is subjected to heavier infection in the beds than a variety like P. O. J. 213, the leaves of which seldom carry more than a trace of red rot at bedding time.

When the cane is taken up in the spring for planting, the cuttings showing evidence of red rot are discarded, but even though extreme care is exercised in the selection of sound cane, the disease may not be detected in some cuttings and subsequent spread of the fungus after planting may result in poor stands. The fact has already been mentioned that losses of bedded seed cane of Cayana may be as high as 50 percent, while destruction of the seed cuttings after planting frequently results in serious stand reductions. P. O. J. 213, on the other hand, which in Louisiana is likewise very susceptible to infection through the root buds and to spread of the disease in the tissues, escaped serious injury prior to 1938. A partial explanation for this may be found in the difference in mass inoculum to which the two varieties are subjected. As will be shown later (p. 59), however, a further explanation is afforded by the difference in relative susceptibility of the two varieties to infection through the root buds to the strains of *Colletotrichum falcatum* present in the sirup-producing areas (see experiment M, p. 57).

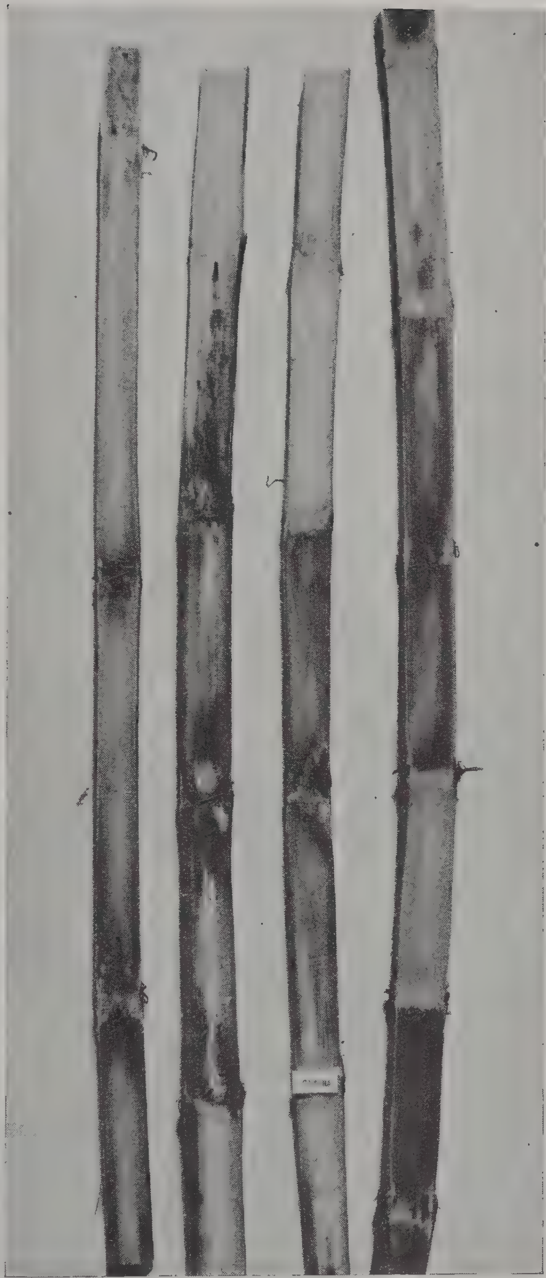


FIGURE 5.—Red rot infection through the nodes of Cayana bedded seed cane from Georgia. Because of its great susceptibility to this type of infection, 50-percent loss from red rot may be sustained in seedbeds of this variety in the sirup districts.

PERSISTENCE OF COLLETOTRICHUM FALCATUM IN THE SOIL

The longevity of *Colletotrichum falcatum* in the soil under field conditions and the role that soil contamination may play in the life history of the organism has not been extensively investigated. Butler (*28*) stated that the fungus can live in the soil or on decaying leaves in the absence of cane, but the evidence indicated that it could not so survive for more than 3 to 4 months. Butler and Hafiz Khan (*30*) found that the fungus died out rapidly in moist soil, but cultures kept dry and exposed to the air retained their vitality for 5 months.

While it seems possible that the fungus may be able to survive for a considerable time on buried trash in the soil and could presumably infect seed cane, this source of inoculum would be relatively unimportant in the United States in view of the mass inoculum to which the seed cuttings are subjected as growing cane, and from the adhering leaves after planting.

Attempts to isolate *Colletotrichum falcatum* directly from the soil have failed, both in the present studies and in a previous survey of the Louisiana soil flora (*6*). It could hardly be expected that the fungus would be obtained by the ordinary methods of plating dilute soil suspensions, because of the relatively small numbers that would exist in the soil in comparison with the common soil-inhibiting saprophytes, which overgrow the plates. A selective medium favoring *C. falcatum* and inhibiting the common saprophytes would be helpful, but such has not been developed.

The best test of the occurrence of *Colletotrichum falcatum* in the soil seemed to be the possibility of obtaining it through the infection of disease-free cuttings of a very susceptible variety. On several occasions in the present study apparently red rot free, surface-sterilized cuttings of P. O. J. 213 were planted in the fall in heavy soil that had been in cane the previous year. These were examined for red rot infection the following spring. Although invaded by species of *Fusarium* and other fungi, none of these cuttings showed typical symptoms of red rot, nor was it possible to isolate the organism from the tissues. These experiments do not justify the conclusion that *C. falcatum* is unable to persist in the soil from one crop year to another, but together with the other evidence presented, they indicate that the soil as a source of inoculum is relatively unimportant in the life history of the organism in Louisiana.

DIRECT INFECTION OF SHOOTS FROM DISEASED CUTTINGS

The divergent opinions regarding the infection of growing shoots by direct mycelial connection with the fungus in diseased cuttings have already been reviewed. Because of the importance of this question in the practical control of the disease, some attention has been given to it in the present investigation.

In an earlier publication (*11*) inoculation experiments in the field designed to determine the effect on germination of inoculating seed cuttings with red rot, were described in detail. In these experiments, cuttings of several commercial varieties and promising seedlings were inoculated in alternate internodes with *Colletotrichum falcatum*, planted in the fall, and examined in the following spring to determine the extent of red rot injury. Observations extending over a period of years were thus made on the spread of the disease in seed cuttings

on several sugarcane varieties. Similar observations have been made of seed cane in commercial fields, with particular attention given to the very susceptible varieties, P. O. J. 213 and C. P. 807. Examinations were made by splitting the shoot longitudinally down through its connection with the seed cutting. Free-hand sections of the connecting tissues were also made for microscopic examination.

In no instance was the red rot fungus observed spreading from the diseased cutting into the shoots arising from the buds thereon. Sometimes the cuttings were so badly rotted that the shoots would die before establishing their own shoot-root systems, an effect due indirectly to the disease through the destruction of the source of nutrients for the young plant, but not to the penetration of the fungus into the tissues of the shoot. As mentioned above, the presence of the fungus could not be demonstrated by either macroscopic or microscopic examination. In view of the rapidity with which it spreads through the parenchyma of very susceptible varieties following infection, it seems improbable that it would be able to develop longitudinally through the vascular bundles from the seed cutting into the shoot without sufficient penetration of the parenchyma occurring as to permit ready detection by macroscopic examination. The author's results confirm those of Edgerton (*33*) in showing that direct mycelial connection between the seed cutting and the growing shoot does not occur in Louisiana, or at least is so rare as to be of no importance in initiating stalk infections.

INFLUENCE OF ENVIRONMENTAL CONDITIONS

TEMPERATURE

After the seed cuttings are planted, soil temperatures have an important bearing on the development of red rot within the stalk. Contrary to what might be expected from the fact that the optimum temperature for *Colletotrichum falcatum* is relatively high (30° to 32° C.), the disease is more destructive to seed cane when soil temperatures following planting are low rather than when they are high. When cane is planted during August, for example, soil temperatures in Louisiana usually fluctuate between 27° and 32° C., which, while near the optium for *C. falcatum*, are sufficiently high to cause quick germination of the sugarcane buds and establishment of independent shoots before red rot has an opportunity to cause severe injury to the cane cutting. While this growth activity on the part of the cane plant may also have a retarding effect on the fungus, it is indirectly a temperature influence.

Fall-planted seed cane usually suffers more severely from red rot than that planted in the summer. During the late fall and winter months, soil temperatures low enough to partly or completely prevent the growth of sugarcane may not so inhibit the red rot fungus. Since the optimum for *Colletotrichum falcatum* is about 5° C. below that for cane, it would be expected that red rot would be able to develop more rapidly than cane at temperatures below the optimum, such as 15° to 20° C., at which temperature the cane remains inactive. Both field observations and laboratory experiments have shown this to be true. It was found, for example, in inoculation experiments with controlled temperatures, that *C. falcatum* would completely rot cuttings of P. O. J. 213 held at 20° C. for 4 to 6 weeks, during which time there was

very little growth activity of the cane as evidenced by swelling of the buds or sprouting of the roots, in either inoculated or uninoculated cuttings.

The close correlation that exists between severe red rot epidemics and winters of low temperature is apparent from an examination of figure 6, in which the mean soil temperatures at Houma, La., for the months of December, January, and February for the winters of 1930–31, 1931–32, 1933–34, and 1935–36 are presented graphically. It will be seen that there were frequent periods during these months when the mean daily soil temperature fell below 60° F. (15.6° C.). At this temperature cane cuttings remain dormant or nearly so, while the red rot fungus is able to develop continuously. The lower

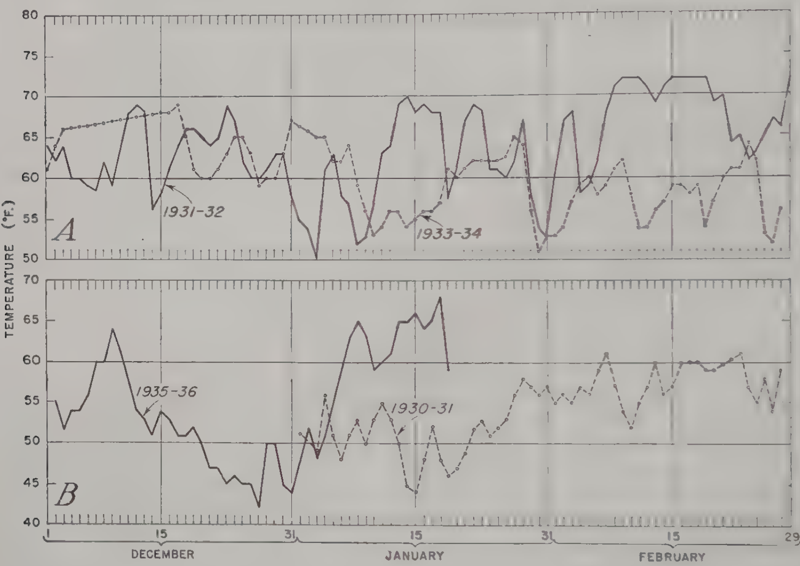


Fig. 6.

FIGURE 6.—Typical differences in mean winter soil temperatures characterizing mild and severe red rot damage to fall planted cane: *A*, Temperatures prevailing during the mild seasons of 1931–32 and 1933–34, showing but brief periods lower than 60° F., contrasted with the curves in *B*, 1930–31 and 1935–36, when red rot damage was severe on susceptible varieties.

limit for the growth of sugarcane is approximately 12° to 14° C. (*59*), depending to some extent on the variety, and growth is very low at 20° C. *Colletotrichum falcatum* grows well at 20° and fairly well at 15° C. The winters of 1930–31 and 1935–36 were severe red rot years, and the records show that they were seasons of abnormally low temperatures. The winters of 1931–32 and 1933–34, on the other hand, were mild, and they were likewise seasons of slight red rot damage to seed cane.

In some seasons, as pointed out by Edgerton and Moreland (*36*), the most destructive development of red rot in seed cane does not occur until the late winter and early spring. Freezing weather in February or March sometimes kills the above-ground portions of shoots that have appeared in periods of warm weather during the

winter, but which have not yet established independent root systems. Then, as warmer weather follows, the red rot spreads very rapidly through the seed cuttings, retarding renewed growth of such shoots, and preventing the germination of many buds, thereby causing serious reductions in the stands of plant cane. A single freeze may destroy all the advance the sugarcane plant has been able to make during the winter, whereas red rot is not so affected. Cold weather may check the spread of the rot, but does not wipe out the progress it has already made.

SOIL MOISTURE

The effect of high soil moisture content in favoring red rot as a seed-cane disease has been mentioned frequently in the literature (*22, 27, 44, 67*). In Louisiana it is a common observation that red rot is most severe on the heavy or "black" lands, which are usually poorly drained. It is on such soils that the most disastrous failure of P. O. J. 213 and the most severe injury to C. P. 807 have occurred. Such conditions favor the disease through the retarding influence they have on the normal growth activities of the sugarcane plant, as well as by the direct favorable effect of high moisture on the fungus.

COMPARATIVE VIRULENCE TESTS OF ISOLATES OF COLLETOTRICHUM FALCATUM

It was evident from the extensive survey of the red rot flora that the races of *Colletotrichum falcatum* present in the United States were of variable morphology, and the constant association of one or the other morphological group with certain sugarcane varieties suggested that differences in virulence might be correlated with these differences in morphology. Experiments were conducted to determine possible differences between groups as well as the range of virulence within groups of the fungus.

LEAF COMPARISONS

Since the disease attacks both leaves and stalks of sugarcane, comparisons of the relative virulence of isolates of the fungus might be made on either. For making such studies on a large scale, comparisons of pathogenicity on leaves seemed to offer some advantages over stalk comparisons. Leaves would be available for such tests during a much greater period of the year than stalks, and a large number of inoculations could be made on leaves with greater economy of time and space than in stalks because of the bulkiness of the latter, the greater time required for making the inoculations, and the necessity for storing them under controlled temperature conditions during the period of incubation. For these reasons, the first comparisons of pathogenicity were made on leaves.

During 1931 several leaf-inoculation tests were conducted with isolates of both morphological races of the fungus, using the commercial varieties of sugarcane and a number of seedlings as hosts, to determine which might be most suitable as differential hosts. The inoculations were made by placing a drop of an aqueous suspension of conidia of *Colletotrichum falcatum* near the center of the leaf midrib, and pricking through this drop four or five times with a sterile insect pin. Only the young fully unfolded upper leaves were inoculated in

order to insure uniformity as to size and age of host material. As a rule, 50 leaves were inoculated with each isolate. Control leaves were similarly treated, using a drop of sterile water instead of the spore suspension. No infection resulted in the controls. After 10 days the length of the lesions was measured and recorded.

On most sugarcane varieties, the leaf midrib lesion produced by *Colletotrichum falcatum* from a single point of inoculation is a continuous one, but frequently on a few varieties and occasionally on most varieties the lesion breaks up into a discontinuous series of red blotches. Microscopic and sometimes macroscopic examination of the apparently healthy intervening tissue may reveal the fungus connecting the blotches of diseased tissue. Such examination requires time, but without it one cannot be sure whether the separate blotches have arisen from one or from several points of inoculation, and in making the measurements of the lesions on the inoculated leaves there was some question whether the measurement should include only the continuous lesion or the entire area of discontinuous blotches. As mentioned earlier, Atkinson and Edgerton's (*19*) work suggests that these separate blotches arise from conidia carried in the transpiration stream. This proved to be one of the difficulties in obtaining reliable results with leaf inoculations.

It soon became apparent also that any differentiation of parasitic races that might be made on the basis of parasitism on leaves would be very different from those based on parasitism in the stalk, since there was found to be no relation between the resistance of a variety to the fungus on the leaves and in the stalk. This may be seen from an examination of the results of a representative leaf-inoculation test which was carried out in the greenhouse, presented in table 7. The leaf lesions on Co. 281, for example, which is highly resistant to the disease in the stalk, averaged longer than those produced by the same isolates on the very susceptible varieties P. O. J. 213, C. P. 807, and D–74 (except in two instances). The average length for the nine light-type isolates was significantly greater on Co. 281 than on any of the susceptible hosts. In this respect the results obtained were contrary to those of Tims and Edgerton (*67*), who found in their leaf inoculation experiments that the rate of spread in leaves of different varieties agreed quite closely with that in the stalks. The average length of the lesions produced on P. O. J. 234 and Co. 281, for example (resistant varieties in the stalk), was less than that on C. P. 807 and P. O. J. 213 (very susceptible in the stalk). Their results indicated that there is a relation between resistance or susceptibility in the stalk and in the leaf.

Both the writer's experimental results and field observations indicate, however, that in general no such relation exists. Under field conditions in Louisiana, P. O. J. 36–M (susceptible) and Co. 281 (resistant) usually show a high percentage of leaf infection by red rot, while the very susceptible varieties P. O. J. 213, D–74, and Louisiana Purple have a low percentage of infection. From observing this condition in the field one might presume that the leaves of the latter varieties were more resistant to the disease than those of the former. The results shown in table 7, however, indicate that this condition is due to the absence of, or resistance to, infection rather than to actual resistance of the tissues to the spread of the fungus, since there was no indication of such resistance when the leaves were artificially

inoculated. It is possible that the lower incidence of red rot lesions on the leaf midribs of the susceptible varieties in the field is due to the fact that they are less attractive to the insects that usually provide the means of entrance for *Colletotrichum falcatum*. As shown in table 7 there is no evidence of marked resistance to the fungus in the leaves of any of the varieties tested. From the results of this test, as well as of others that have been conducted, it appears that leaves of sugarcane varieties do not exhibit the marked differences in susceptibility to *C. falcatum* shown by the stalks. That some varieties are more susceptible than others, however, seems obvious from field observations in which it has been noted that the lesions on the leaves of some varieties, such as C. P. 28/19 and Co. 290, are much longer than those on P. O. J. 213. Lesions extending the full length of the midrib have never been observed on the latter variety, whereas they are common on the two former when growing in adjacent fields under identical environmental conditions (plate 2, *A* and *B*).

TABLE 7.—*Average length of lesions produced by 11 light-race and 5 dark-race isolates of Colletotrichum falcatum on leaf midribs of several varieties of sugarcane*

[10 days' incubation; means of 50 replications]

LIGHT-RACE ISOLATES

Isolate No.	Mean length of midrib lesions						
	P. O. J. 36–M	P. O. J. 213	Co. 281	C. P. 807	D–74	Louisiana Purple	MEAN
	Cm	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	
L–1	11.1	11.4	13.7	7.9			11.1
L–2	12.0	12.7		9.2			11.3
L–6	30.3	23.8	26.5	17.0			24.4
L–7		33.1	28.3	15.6	5.5		20.6
L–9	21.2	22.8	25.3	14.9	18.6		20.6
L–10	9.4	12.6		12.3	18.5		13.2
L–31	13.3	14.1	12.2	9.9	5.7		10.6
L–33	16.0		16.0		11.3	10.0	14.4
L–35	14.8		20.0		15.8	13.8	16.1
L–56	12.2	13.1	16.5	8.0	17.5	13.1	13.4
L–71	10.3	9.6	12.8	7.4	8.7		9.8
Average of light races	15.1	17.0	19.0	11.4	12.7	12.3	

DARK-RACE ISOLATES

G–68	8.5	8.0		9.1	5.0		7.6
G–72	12.0	11.6	9.4	10.5			11.9
G–78	8.7			11.4			10.0
G–82	7.4	12.7	9.8	4.2	16.4	10.8	10.2
G–83	7.1			7.1	3.7		[illegible]
Average of dark races	8.7	10.8	9.6	8.5	8.4	10.8	
	12.8	15.4	17.3	10.3	11.5	11.9	

The figures in table 7 indicate that the dark-race isolates are less virulent on leaves of the sugarcane varieties tested than the light races. This is worthy of note in view of the similar results obtained in the stalk inoculation tests which are described on p. 50.

Even though parasitic races of *Colletotrichum falcatum* might be differentiated on the basis of their parasitism on leaves of a series of differential hosts, it soon became apparent that the races so differentiated might be very different from those differentiated on the

basis of parasitism in the stalks, and would thus bear no relation to the failure of sugarcane varieties due to red rot. The leaf inoculations were therefore discontinued. Furthermore, as pointed out by Tims and Edgerton (*67*), the great variation between the length of lesion on different leaves of the same variety tend to make the leaf inoculations less reliable than those in the stalks, from which more uniform results can be obtained. The leaf inoculations, however, brought out two points: (1) That although consistent differences are seen in nature, there is less marked difference in resistance or susceptibility to red rot in the leaves from artificial inoculation of different sugarcane varieties such as is exhibited by the stalks, none having been found that could be classed as resistant; and (2), they gave the first indication of differences in virulence between the light and dark morphological races of the fungus.

STALK COMPARISONS

The comparisons of the relative virulence of isolates of *Colletotrichum falcatum* in the stalks were made by inoculating mature stalks with the various isolates of the fungus, incubating them for a certain period, and then measuring the spread of the fungus laterally and longitudinally within the stalk.

HOST VARIETIES

Several preliminary experiments were conducted to determine which varieties would be most suitable for such comparisons. These included the very susceptible Louisiana Purple, D–74, P. O. J. 213, and C. P. 807, the susceptible P. O. J. 36–M, and the resistant Co. 281 and P. O. J. 234. Among the susceptible canes, the Purple and D–74 proved unsuitable not only because of heavy borer infestation and the high percentage of natural infection by *Colletotrichum falcatum*, but also because their large stalk diameter and consequent bulk made them inconvenient to handle in large quantities. C. P. 807 had the disadvantage of a very hard rind, which made it difficult to punch the holes rapidly for making the artificial inoculations. Because of relatively low borer infestation and resistance to root ring infection by *C. falcatum*, however, the stalks usually show relatively little natural infection.

P. O. J. 213 was finally selected as the most suitable among the susceptible hosts because of the relative softness of the rind, long internodes, which permitted the fungus considerable spread before being retarded by the nodes, and the small diameter, which permitted economy of storage space. In the sugar district of southern Louisiana, however, P. O. J. 213 usually suffers heavily from borer infestation, and even when not bored may show considerable natural infection by red rot through the root rings. To obtain suitable material for the inoculations, therefore, it was necessary to go to north-central Louisiana, where both the borer and red rot are not important, and where cane could be obtained in large quantities that could be rendered relatively free of natural infection by surface disinfection. Co. 281 was chosen as the most suitable resistant host.

CHOICE OF ISOLATES

The number of isolates used in any one test was necessarily limited to about 50 by the space available for storing the inoculated cuttings, as well as by the supply of red rot-free cuttings which could be obtained conveniently at one time for such tests. This meant the comparison of relatively small samples of the red rot populations from any one host variety or locality. Since there was no way of knowing which isolates were most representative of the populations in nature, the choice of cultures for the tests was made at random. This was done by placing in baskets the total number of culture tubes from the varieties or localities to be compared, and then taking them out at random until the number to be used in the test had been obtained. Where it was desired to include certain individual cultures in the tests, the reason for their choice is explained in the discussion of the tests in which they were used. The results would doubtless be more reliable if larger samples of the populations could have been compared, but in view of the fairly liberal sampling of the fields studied, their widespread geographic distribution, and the random selection of isolates from the total number obtained, it is believed that the isolates included in the tests are reasonably representative of the red rot population as it occurs in nature.

METHOD OF INOCULATION

The stalks of cane were prepared for the tests by removing the sheaths and tops, cutting into 4-foot lengths, washing, and immersing in a solution of formaldehyde, 1:240, for 2 hours, to eliminate conidia of *Colletotrichum falcatum* that might be adhering to the surface. When dry, holes were punched in the center of two internodes of each cutting, leaving at least one and, where possible, two internodes between those punched. A specially devised punch similar to a cork borer with an opening 2.5 mm in diameter was used.

With a sterile instrument a piece of a 15- to 20-day-old culture of the *Colletotrichum* on oatmeal agar was inserted into these holes. The inoculated cuttings were tied in bundles of 10 each and incubated for 20 to 30 days at 20° to 25° C., after which they were split lengthwise and the spread of red rot measured. The results were expressed as the "index of virulence," which was obtained by multiplying the ratio of lesion width to stalk diameter by the ratio of lesion length to internode length.

COMPARATIVE VIRULENCE ON A SUSCEPTIBLE HOST, P. O. J. 213

Test F

The purpose of test F was to compare the virulence of the light and dark races of *Colletotrichum falcatum* on the very susceptible variety P. O. J. 213, in order to determine whether there was a correlation between virulence and the morphological characters differentiating the two cultural races. Evidence of such a correlation, i. e., high virulence with light color of mycelium, would offer support to the hypothesis that the failure of this variety in Louisiana resulted from a change in the dominant red rot flora in the State, from the dark to the light races, coincident with its widespread commercial utilization. In addition, the inclusion of groups of light-race isolates from P. O. J. 213

and other varieties offered an opportunity to compare the virulence of the various groups, to determine whether there was evidence of parasitic specialization of the P. O. J. 213 isolates on that variety.

Fifty-five isolates were used in this test, as follows: 8 from P. O. J. 213, representing 6 localities; and 7 from Co. 281, representing 3 localities, in the 3 geographic areas of the sugar district; 8 from P. O. J. 36-M, representing 2 localities in the northern parishes where this variety was extensively grown; 9 from D-74, from 2 localities in Terrebonne Parish; and 9 from miscellaneous varieties, representing 4 localities in the 3 geographic subdivisions of the sugar district. Of the 14 dark-race isolates used, 8 were from Louisiana, representing 4 localities; and 6 were from Georgia, representing 3 localities in the southern part of that State.

The results, which are the means of 16 readings for each isolate, appear in table 8 and the frequency distribution according to viru-

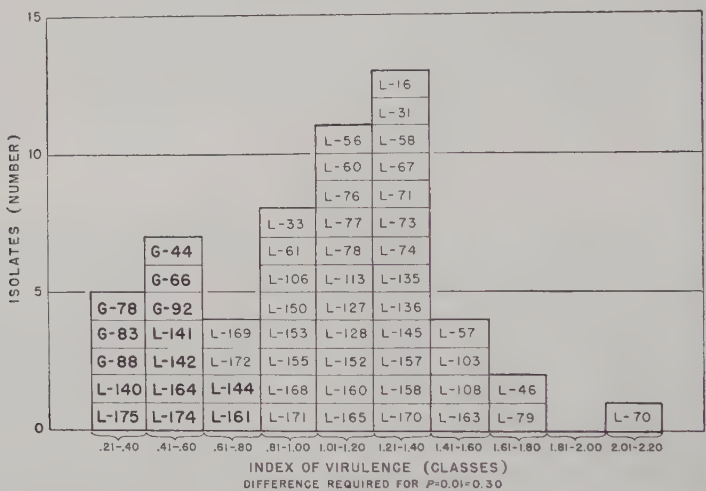


FIGURE 7.—Frequency distribution of 55 isolates representing both light and dark morphological groups of *Colletotrichum falcatum* arranged according to the mean virulence index on P. O. J. 213. (Dark-race isolates in boldface type.)

lence classes in figure 7. In the figure, the culture numbers of the dark-colored isolates appear in boldface type to distinguish them from the light ones.

It may be seen from table 8 and the frequency distribution that, considered as groups, the light-race isolates from Louisiana (group A) were 57.1 percent more virulent on P. O. J. 213 than the dark ones from Louisiana (group B), and 67.2 percent more virulent than the dark ones from Georgia (group C), differences which are highly significant. These results, which indicate that dark color of mycelium and low virulence in *Colletotrichum falcatum* are correlated characters, attach added importance to the survey of the red rot flora, which showed that the light-race isolates were responsible for the failure of P. O. J. 213 in Louisiana; and when considered in the light of the fact that the dark cultural race is dominant in Georgia indicate that the absence of red rot injury to the variety there prior to 1938 may

have been due to the absence of the more virulent light races which caused its failure in Louisiana.

TABLE 8.—*Average virulence index on P. O. J. 213 of groups of light-race isolates of Colletotrichum falcatum from different varieties in Louisiana compared with 8 dark-race isolates from Louisiana and 6 from Georgia (test F)*

[Virulence index of each isolate based on measurement of 16 lesions in inoculated stalks after 28 days' incubation at 20° to 22° C.]

Group and isolate No.	Source		Virulence index	Group difference
	Variety	Plantation or locality		
Group A:				*Percent*
Light-race isolates from Louisiana:				
Subgroup A-1:				
L-46	P. O. J. 213	Southdown	1.75	
L-56	do	Armant	1.01	
L-77	do	Lafayette	1.11	
L-78	do	do	1.13	
L-79	do	New Iberia	1.69	
L-103	do	Camperdown	1.48	
L-106	do	Jeanerette	.94	
L-108	do	do	1.45	
Mean of 8 isolates			1.32	
Subgroup A-2:				
L-57	P. O. J. 36-M	Alma	1.41	
L-58	do	do	1.25	
L-60	do	do	1.02	
L-61	do	do	.84	
L-70	do	Ashland (Bunkie)	2.06	
L-71	do	do	1.27	
L-73	do	do	1.26	
L-74	do	do	1.26	
Mean of 8 isolates			1.30	
Percent less virulent than subgroup A-1.				1.5
Percent required for $P=0.01$.				8.3
Subgroup A-3:				
L-31	Co. 281	Southdown	1.29	
L-67	do	Ashland (Bunkie)	1.39	
L-135	do	Southdown	1.31	
L-136	do	do	1.36	
L-145	do	do	1.27	
L-168	do	Billeaud	.93	
L-169	do	do	.70	
Mean of 7 isolates			1.18	
Percent less virulent than subgroup A-1.				10.6
Percent required for $P=0.01$.				8.3
Subgroup A-4:				
L-127	D-74	Southdown	1.12	
L-128	do	do	1.17	
L-150	do	do	.88	
L-152	do	do	1.02	
L-153	do	do	.98	
L-155	do	do	.86	
L-157	do	Bayou du Large (Houma)	1.33	
L-158	do	do	1.27	
L-160	do	do	1.15	
Mean of 9 isolates			1.09	
Percent less virulent than subgroup A-1.				17.4
Percent required for $P=0.01$.				7.6

TABLE 8.—*Average virulence index on P. O. J. 213 of groups of light-race isolates of Colletotrichum falcatum from different varieties in Louisiana compared with 8 dark-race isolates from Louisiana and 6 from Georgia (test F)*—Continued

Group and isolate No.	Source: Variety	Source: Plantation or locality	Virulence index	Group difference
				Percent
Group A—Continued.				
Light-race isolates from Louisiana—Continued.				
Subgroup A-5:				
L-16	C. P. 807	Southdown	1.26	
L-33	Louisiana Purple	do	.90	
L-76	Louisiana Striped	Ashland (Bunkie)	1.17	
L-113	Cayana	Hollywood	1.01	
L-163	Co. 290	Billeaud	1.56	
L-165	do	do	1.08	
L-170	C. P. 28/11	do	1.36	
L-171	Co. 290	Southdown	.84	
L-172	do	do	.75	
Mean of 9 isolates			1.10	
Percent less virulent than subgroup A-1.				16.7
Percent required for $P=0.01$.				7.6
Group A, weighted mean			1.19	
Group B:				
Dark-race isolates from Louisiana:				
L-140	P. O. J. 234	Woodlawn (Houma)	.36	
L-141	do	do	.43	
L-142	do	do	.48	
L-144	Co. 281	Southdown	.66	
L-161	D-74	Bayou du Large (Houma)	.61	
L-164	Co. 290	Billeaud	.60	
L-174	do	Southdown	.52	
L-175	do	do	.39	
Mean of 8 isolates			.51	
Percent less virulent than group A.				57.1
Percent required for $P=0.01$.				6.7
Group C:				
Dark-race isolates from Georgia:				
G-44	Cayana	Farm near Cairo	.46	
G-66	P. O. J. 213	U. S. Sugar Plant Field Laboratory, Cairo, Ga.	.42	
G-78	P. 807	do	.33	
G-83	Cayana	Quitman	.30	
G-88	do	Farm near Cairo	.35	
G-92	do	do	.45	
Mean of 6 isolates			.39	
Percent less virulent than group A.				67.2
Percent required for $P=0.01$.				7.6

The eight cultures from P. O. J. 213 (subgroup A-1) proved to be significantly more virulent than those from the other varieties, with the exception of the group from P. O. J. 36-M (A-2). The high average index for the latter group was due to the exceptionally high reading for the culture L-70. The highly significant differences between the isolates from P. O. J. 213 and those from Co. 281, D-74, and the miscellaneous varieties (and for those from P. O. J. 36-M without L-70) may be interpreted as evidence of parasitic specialization of the former group on that variety. More conclusive evidence might have been obtained if a much larger number of isolates from

P. O. J. 213 and the other varieties had been tested. Such might have been revealed also if cultures of the fungus from the period prior to the widespread expansion of P. O. J. 213 had been available. It is possible that at the time these cultures were obtained, the multiplication of the race or races causing the failure of P. O. J. 213 on this variety, and subsequent dissemination to other commercial varieties, had resulted in the dominance of these races on other varieties. Consequently, at least some of the isolates obtained from the varieties other than P. O. J. 213 may have originated on that variety.

Test J

Test J was conducted primarily to determine whether the dark-race isolates are a uniform group parasitically, or whether they exhibit a range of virulence on P. O. J. 213 similar to that shown by the light-race isolates in test F. A comparison of the isolates from Cayana and P. O. J. 213 from the sirup-producing States seemed of particular interest also because of the severe red rot injury suffered by the former variety and the absence of injury to the latter in those States. The 52 isolates used in the test included 20 dark-type cultures from Louisiana, representing 9 widely separated localities in the sugar district; 25 dark-type isolates from localities in Alabama, Georgia, and Mississippi; and 7 light-type isolates from Florida, Georgia, Mississippi, and Louisiana, which were included in order to compare the 2 cultural races. The results appear in table 9 and figure 8.

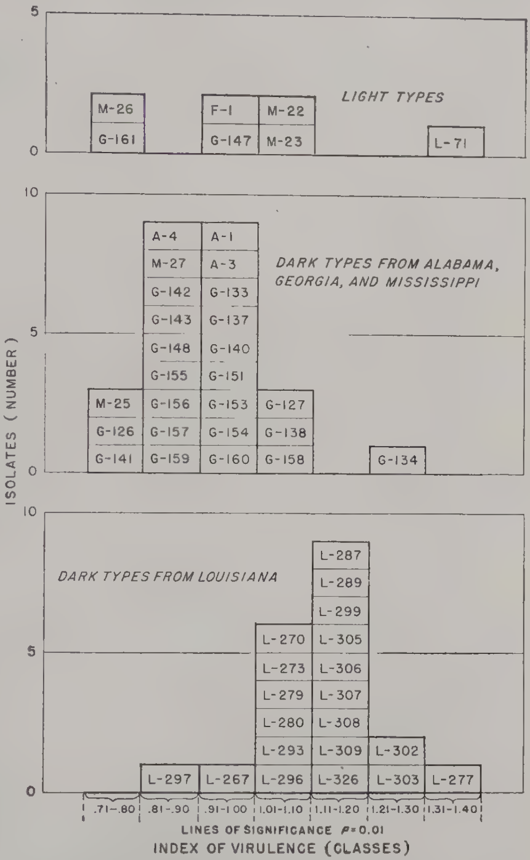


FIGURE 8.—Frequency distribution of 52 isolates of *Colletotrichum falcatum* according to the index of virulence on P. O. J. 213, in test J.

It is apparent from these results that the dark-race isolates exhibited a range of virulence similar to that shown by the light-type isolates in test F. Those from Louisiana (group A) were significantly more virulent (18.6 percent) than those from the sirup-producing States (group B), and both groups were less virulent than the single Louisi-

ana light-type culture, L–71, included in the test. This isolate, which in test F was intermediate in virulence among the light types, proved in test J to be 22 percent more virulent than the Louisiana dark types and 50 percent greater than those from the sirup-producing States. For the Louisiana isolates, the relationship between the two cultural races with respect to virulence is thus seen to be the same as that in test F. For the sirup-producing States, the isolates of the two cultural races were approximately equal in virulence.

TABLE 9.—*Average virulence index on P. O. J. 213 of a group of dark-race isolates and one light-race isolate from Louisiana compared with groups of light- and dark-race isolates from the sirup-producing States (test J)*

Virulence index of each isolate based on measurements of lesions in 20 inoculated stalks after 28 days' incubation at 20° to 22° C.]

Group and isolate No.	Source		Virulence index	Group difference
	Variety	Plantation or locality		
Group A:				
Dark-race isolates from Louisiana:				
L–267	C. P. 28/19	Cinclare	1.00	
L–270	Co. 281	Alma	1.08	
L–273	P. O. J. 36–M	Albania	1.07	
L–277	C. P. 807	Hollywood	1.31	
L–279	do	Greenwood	1.10	
L–280	do	do	1.03	
L–287	Co. 281	Cinclare	1.17	
L–289	C. P. 28/19	Lafayette	1.17	
L–293	Co. 281	Port Allen	1.04	
L–296	do	Cinclare	1.10	
L–297	P. O. J. 234	Raceland	.84	
L–299	Co. 281	do	1.16	
L–302	P. O. J. 213	do	1.27	
L–303	P. O. J. 36–M	Greenwood	1.24	
L–305	Co. 290	do	1.18	
L–306	do	do	1.18	
L–307	do	do	1.16	
L–308	do	do	1.19	
L–309	Johnson grass	do	1.16	
L–326	P. O. J. 36–M	Glenwild	1.11	
Mean of 20 isolates			1.13	
Group B:				
Dark-race isolates from Alabama, Georgia, and Mississippi:				
Subgroup B–1:				
A–1	Cayana	Dothan, Ala	1.00	
A–3	do	do	.97	
G–126	do	10 miles south of Cairo, Ga	.80	
G–127	do	do	1.02	
G–137	do	Cairo, Ga.[1]	.98	
G–138	do	do	1.03	
G–140	do	do	.93	
G–141	do	do	.76	
G–142	do	do	.86	
G–151	do	Bainbridge, Ga	.96	
G–153	do	do	.94	
G–154	do	do	1.00	
Mean of 12 isolates			.94	
Subgroup B–2:				
A–4	P. O. J. 213	Luverne, Ala	.85	
G–134	do	Cairo, Ga.[1]	1.28	
G–155	do	do	.81	
G–156	do	do	.89	
G–157	do	do	.87	
G–158	do	do	1.02	
G–159	do	do	.86	
G–160	do	do	.97	
M–25	do	Poplarville, Miss	.80	
M–27	do	Laurel, Miss	.84	
Mean of 10 isolates			.92	

[1] From different farms in the vicinity of Cairo, Ga.

TABLE 9.—*Average virulence index on P. O. J. 213 of a group of dark-race isolates and one light-race isolate from Louisiana compared with groups of light- and dark-race isolates from the sirup-producing States (test J)*—Continued

Group and isolate No.	Source: Variety	Source: Plantation or locality	Virulence index	Group difference
Group B—Continued.				
Dark-race isolates from Alabama, Georgia, and Mississippi—Con.				*Percent*
Subgroup B-3:				
G-133	Co. 281	Cairo, Ga	0.98	
G-143	Co. 515	do	.85	
G-148	Co. 281	do	.84	
Mean of 3 isolates			.89	
Weighted mean of 25 isolates.			.92	
Percent less virulent than group A.				18.6
Percent required for $P=0.01$.				3.5
Group C:				
Light-race isolates from Florida, Georgia, and Mississippi:				
F-1	P. O. J. 213	Homestead, Fla	.91	
G-147	Co. 281	Cairo, Ga	.95	
G-161	P. O. J. 213	10 miles south of Cairo, Ga.	.79	
M-22	Co. 290	Poplarville, Miss	1.08	
M-23	Cayana	do	1.01	
M-26	P. O. J. 213	Laurel, Miss	.74	
Mean of 6 isolates			.91	
Percent less virulent than group A.				19.5
Percent required for $P=0.01$.				5.3
Group D:				
Light-race isolate from Louisiana:				
L-71	P. O. J. 36-M	Ashland (Bunkie)	1.38	
Mean			1.38	
Percent more virulent than group A.				22.1
Percent required for $P=0.01$.				11.5

Within group B (sirup-producing States dark types) the isolates from P. O. J. 213 and Cayana were approximately equal in virulence, while the isolate L-71, which in test F was found to be intermediate in virulence among the Louisiana light types, proved in this test to be 50 percent more virulent than the isolates of group B. Assuming that L-71 is representative of the Louisiana light-race flora (and numerous inoculation tests have indicated that it is), and that the isolates of group B are representative of the red rot population of the sirup-producing States, the results suggest that a partial explanation for the absence of red rot injury to P. O. J. 213 in those States prior to 1938 lies in the lower virulence toward it of the races of *Colletotrichum falcatum* predominant there.

TEST M

The object of test M was to compare the virulence of isolates of *Colletotrichum falcatum* from Louisiana and the sirup-producing States with respect to their ability to infect P. O. J. 213 through the root

primordia. It was felt that since root ring infection of this variety is rare in the sirup-producing States, the information gained from such a test might aid in explaining its freedom from red rot injury for many years in that region.

METHOD OF INOCULATION

Twelve isolates were used in the test as follows: Two light-race isolates from Louisiana; three light-race isolates from Georgia; and seven dark-race isolates from Georgia, Alabama, and Florida. Cuttings of P. O. J. 213 were obtained from Cairo, Ga. They were stripped clean of trash in the field, cut into about 3-foot lengths, and treated with hot water at 50° to 52° C. for 20 minutes before being transported to Houma, La. It seemed that the complete freedom from borer injury and the light infection of red rot on the leaves in the field should render them fairly free of natural red rot infection.

On arrival at Houma they were divided into 13 lots of 20 cuttings each, and over the nodes of each lot was smeared an oatmeal-agar culture of one of the isolates of *Colletotrichum falcatum*. One lot was left without inoculation as a control. Each lot of cuttings was then covered with moist, steamed soil in a flat, 3½ feet long and 8 inches deep, and placed under a shed outdoors. About 3 inches of fresh sawdust was placed under the flats and a covering of about 6 inches was placed over the top for the purpose of preventing contamination from the outside, to provide insulation against rapid fluctuations of temperature, and to prevent drying out. The temperature in one of the flats was recorded by means of a soil thermograph. The temperature was 60° F. at the beginning of the experiment on December 15, 1936, and remained between 60° and 70° until January 10. From that date until February 3 it varied between 70° and 80°, and then declined to between 58° and 62° for the remainder of the experiment. The cuttings were inoculated on December 15, 1936, and left undisturbed until March 14, 1937, when they were taken up, split lengthwise, and examined for red rot infection. From each node that showed indications of red rot infection a piece of tissue was cultured in acidified oatmeal agar.

Relative infection of P. O. J. 213 through the root primordia by various isolates of *Colletotrichum falcatum* from inoculum smeared over the nodes after 2 months' incubation ranging from 60°–80° F. (approximately 60 nodes inoculated with each isolate) is shown as follows:

Isolate number:	Number of nodes infected (*Colletotrichum* reisolated)
Light types from Louisiana:	
L–71	12
L–103	4
Light types from Georgia:	
G–104	1
G–161	0
G–164	0
Dark types from Alabama, Georgia, and Florida:	
G–111	0
G–167	2
G–168	0
G–170	0
G–173	3
A–4	0
F–23	0

RESULTS

It will be noted that *Colletotrichum falcatum* was recovered from cuttings inoculated with five of the isolates, two of them dark types from Georgia, one light type from Georgia, and the two light types from Louisiana. This indicated that under the conditions of the experiment, in which the cuttings were given rather extreme treatment with respect to inoculation and favorable conditions for infection, certain isolates of both morphological races of the fungus were capable of infecting P. O. J. 213 through the root buds. But of particular significance was the fact that the Louisiana light-type isolate L–71 produced infection in 12 nodes, compared with 2 cases of infection with the Georgia dark type G–167 and 3 cases with G–173. Four nodes were infected by L–103, another light type from Louisiana. Considerable spread of the fungus through the stalks had resulted with L–71, while little spread had occurred with the other isolates. While the results of this experiment cannot be taken as conclusive proof, they indicate that P. O. J. 213 is more susceptible to nodal infection by certain Louisiana light types (as represented by L–71) than to the dark types of the sirup-producing States.

COMPARATIVE VIRULENCE ON A RESISTANT HOST, CO. 281

Test K

Tests similar to F and J were conducted with Co. 281 as the host to determine whether the same relationship between the light and dark races as found in those tests would occur on a resistant variety. This proved to be the case. Of the several such tests carried out on Co. 281, test K, conducted in 1935, is presented to illustrate this difference, as well as to afford an opportunity of comparing the relative virulence of the dark races, which the survey showed were rapidly increasing at that time in Louisiana. As already pointed out, the survey had indicated that the predominance of the dark races on Co. 281 leaf lesions was one factor in the increasing population of this group in the State, and it seemed important to determine whether any forms virulent in stalks of Co. 281 were represented in this increasing population.

Twenty-seven light-race and 42 dark-race isolates were used in the test. Of the light races, 12 were from Louisiana. Five of these were from Co. 281, each representing a different locality in the northern and southeastern parishes. One of these isolates, L–31, was obtained from severely rotted seed cane of Co. 281 at Houma, La., and was included because several preliminary tests had indicated that it possessed a high degree of virulence toward that variety. Seven of the light races were from miscellaneous commercial varieties, three from the northern parishes, and two from each of the other geographic areas of the sugar district. Of the 15 light races from Florida, Georgia, and Mississippi (group B), 6 were from 2 localities in southern Florida, 3 from 2 localities in Georgia, and 6 from 3 localities in southern and central Mississippi.

Of the 42 dark-race isolates, the 12 from Co. 281 represented 8 localities in the 3 geographic subdivisions of the State; the 8 from C. P. 807 represented 4 localities where severe red rot injury had been suffered by this variety; and the 10 isolates from the miscellaneous varieties represented 5 localities in the western and southeastern

parishes. The dark races from the sirup-producing States represented two localities in Alabama, four in Georgia, three in Mississippi, and one in Texas. Thus, it seemed that the distribution of the isolates of both morphological races used in the test, with respect to host variety and geographic localities, was such as to insure a fairly representative sampling of the red rot population. The results of the test are presented in table 10 and figure 9.

The distribution of light- and dark-race isolates with respect to virulence (fig. 9) is similar to that in test F. As would be expected on a resistant host, however, there was a sharper demarcation between those of high and low virulence, the isolates of relatively low virulence having a median considerably to the left of that of the former test. There was also a tendency for the light-race isolates from southern Florida and several from the sirup-producing States to fall with the dark ones from these States.

The mean virulence index of the five Co. 281 light-race isolates (subgroup A–1) was significantly greater than that for those from other varieties (subgroup A–2), but this difference was due entirely to the high reading for L–31. The remaining Co. 281 isolates tested (subgroup A–1) were of about the same virulence as those from the other varieties.

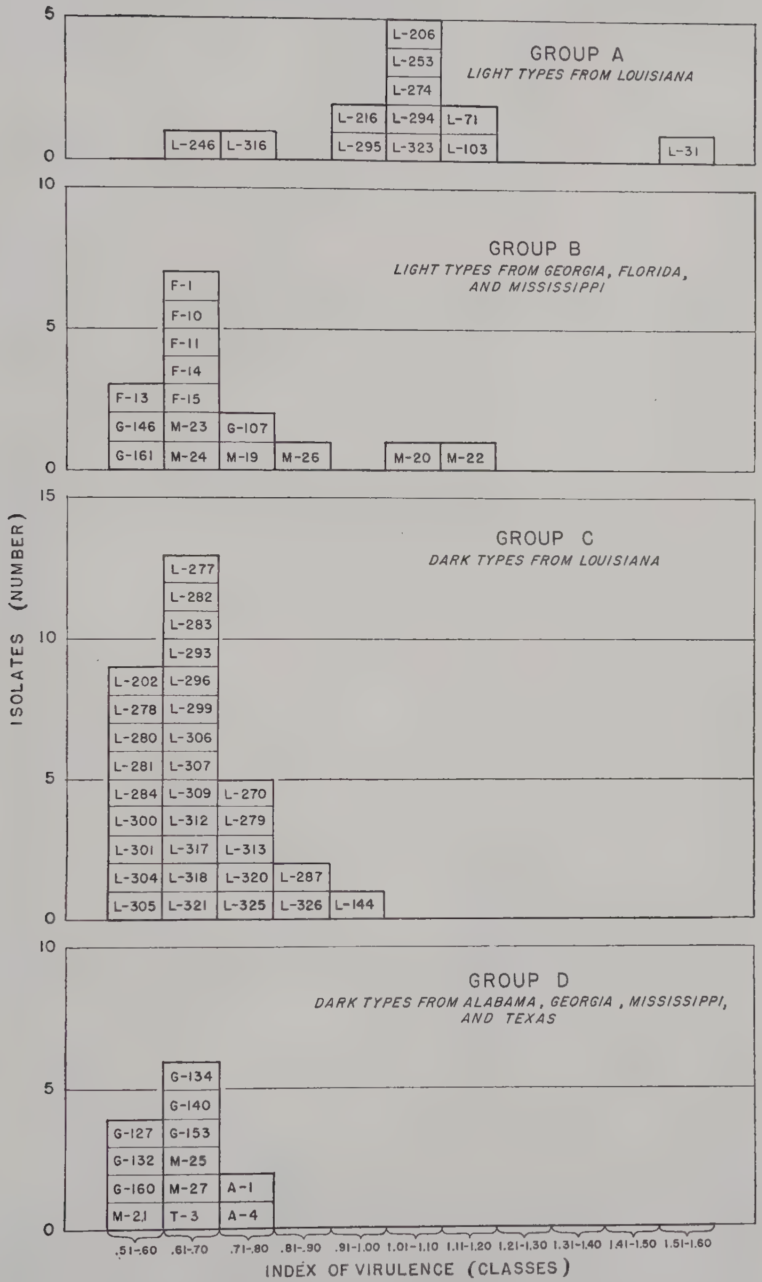


FIGURE 9.—Frequency distribution of groups of dark- and light-race isolates of *Colletotrichum falcatum* from different varieties and geographic localities, arranged according to their index of virulence on the resistant variety Co. 281 (test K).

TABLE 10.—*Average virulence index on the resistant Co. 281 variety of groups of light- and dark-race isolates of Colletotrichum falcatum from different varieties and States compared with a representative light-race group from Louisiana (test K)*

[Virulence index of each isolate based on measurements of lesions in 40 inoculated stalks after 30 days' incubation at 20°–22° C.]

Group and isolate No.	Source		Virulence index	Group difference
	Variety	Plantation or locality		
Group A:				*Percent*
Light-race isolates from Louisiana:				
Subgroup A–1:				
L–31	Co. 281	Southdown	1.54	
L–253	do	La Place	1.10	
L–294	do	Port Allen	1.07	
L–295	do	Cinclare	.94	
L–323	do	Crescent Farm	1.08	
Mean of 5 isolates			1.15	
Subgroup A–2:				
L–71	P. O. J. 36–M	Bunkie (Ashland)	1.19	
L–103	P. O. J. 213	Camperdown	1.20	
L–206	do	Washington	1.01	
L–216	D–74	Bayou du Large (Houma)	.96	
L–246	C. P. 29/320	Albania	.70	
L–274	P. O. J. 213	Cedar Grove	1.07	
L–316	P. O. J. 36–M	Crescent Farm	.77	
Mean of 7 isolates			.99	
Percent less virulent than subgroup A–1.				13.9
Percent required for $P=0.01$				6.1
Group A, weighted mean			1.05	
Group B:				
Light-race isolates from Florida, Georgia, and Mississippi:				
F–1	P. O. J. 213	Homestead, Fla	.65	
F–10	P. O. J. 2714	Clewiston, Fla	.70	
F–11	do	do	.65	
F–13	do	do	.60	
F–14	do	do	.66	
F–15	do	do	.64	
G–107	Louisiana Purple	Cairo, Ga	.74	
G–146	Co. 281	do	.55	
G–161	P. O. J. 213	South of Cairo, Ga	.58	
M–19	C. P. 29/116	Poplarville, Miss	.77	
M–20	C. P. 28/19	do	1.07	
M–22	Co. 290	do	1.13	
M–23	Cayana	do	.70	
M–24	P. O. J. 213	2 miles south of Poplarville, Miss.	.64	
M–26	do	Near Laurel, Miss	.86	
Mean of 15 isolates			.73	
Percent less virulent than group A.				30.5
Percent required for $P=0.01$				4.8
Group C:				
Dark-race isolates from Louisiana:				
Subgroup C–1:				
L–144	Co. 281	Southdown	.96	
L–270	do	Alma	.72	
L–287	do	Cinclare	.83	
L–293	do	Port Allen	.84	
L–296	do	Cinclare	.61	
L–299	do	Raceland	.67	
L–300	do	do	.55	
L–301	do	do	.56	
L–304	do	Greenwood	.57	
L–312	do	Crescent Farm	.67	
L–320	do	Bayou du Large (Houma)	.72	
L–321	do	do	.70	
Mean of 12 isolates			.68	

TABLE 10.—*Average virulence index on the resistant Co. 281 variety of groups of light- and dark-race isolates of Colletotrichum falcatum from different varieties and States compared with a representative light-race group from Louisiana (test K)*—Continued.

Group and isolate No.	Source: Variety	Source: Plantation or locality	Virulence index	Group difference
Group C—Continued.				*Percent*
Dark-race isolates from Louisiana—Continued.				
Subgroup C-2:				
L-277	C. P. 807	Hollywood	0.67	
L-278	do	Raceland	.60	
L-279	do	Greenwood	.72	
L-280	do	do	.53	
L-281	do	do	.60	
L-282	do	do	.64	
L-283	do	do	.68	
L-284	do	Albania	.58	
Mean of 8 isolates			.63	
Subgroup C-3:				
L-202	Louisiana Purple	Franklin	.59	
L-305	Co. 290	Greenwood	.54	
L-306	do	do	.62	
L-307	do	do	.62	
L-309	Johnson grass	do	.62	
L-313	C. P. 28/19	Crescent Farm	.71	
L-317	P. O. J. 213	Bayou du Large (Houma)	.69	
L-318	do	do	.70	
L-325	P. O. J. 36-M	Glenwild	.73	
L-326	do	do	.86	
Mean of 10 isolates			.67	
Weighted mean, group C			.66	
Percent less virulent than group A.				37.1
Percent required for $P=0.01$				3.8
Group D:				
Dark-race isolates from Alabama, Georgia, Mississippi, and Texas:				
A-1	Cayana	Dothan, Ala	.71	
A-4	P. O. J. 213	Luverne, Ala	.76	
G-127	Cayana	10 miles south of Cairo, Ga.	.59	
G-132	Co. 281	Cairo, Ga	.56	
G-134	P. O. J. 213	do	.66	
G-140	Cayana	do	.67	
G-153	do	Bainbridge, Ga	.63	
G-160	P. O. J. 213	Near Cairo, Ga	.59	
M-21	C. P. 28/19	Poplarville, Miss	.57	
M-25	P. O. J. 213	South Poplarville, Miss	.64	
M-27	do	10 miles north of Laurel, Miss.	.70	
T-3	Cayana	Silsbee, Tex	.67	
Mean of 12 isolates			.65	
Percent less virulent than group A.				38.1
Percent required for $P=0.01$				4.8

Among the Louisiana dark-race isolates (group C), the 12 from Co. 281 and the 8 from C. P. 807 were approximately equal to those from the other varieties. The general mean index for the dark-race isolates from Louisiana was approximately the same as that for those from the sirup-producing States, which contrasts with the significant differences obtained in test J conducted on the very susceptible P. O. J. 213 variety.

The test brings out strikingly the high virulence of the culture L-31 on Co. 281. This is illustrated in plate 8, where the extensive rotting

by L–31 is contrasted with the milder infections produced by representatives typifying the different geographic collections of the fungus. The mean virulence index of this isolate was 28 percent greater than that of the next most virulent one studied (L–103), and 34 percent greater than the general mean of the other light types included in the test. On P. O. J. 213 (test F), on the other hand, it fell only at the midpoint in the virulence scale, which in conjunction with the above suggests that it is differentially adapted to Co. 281.

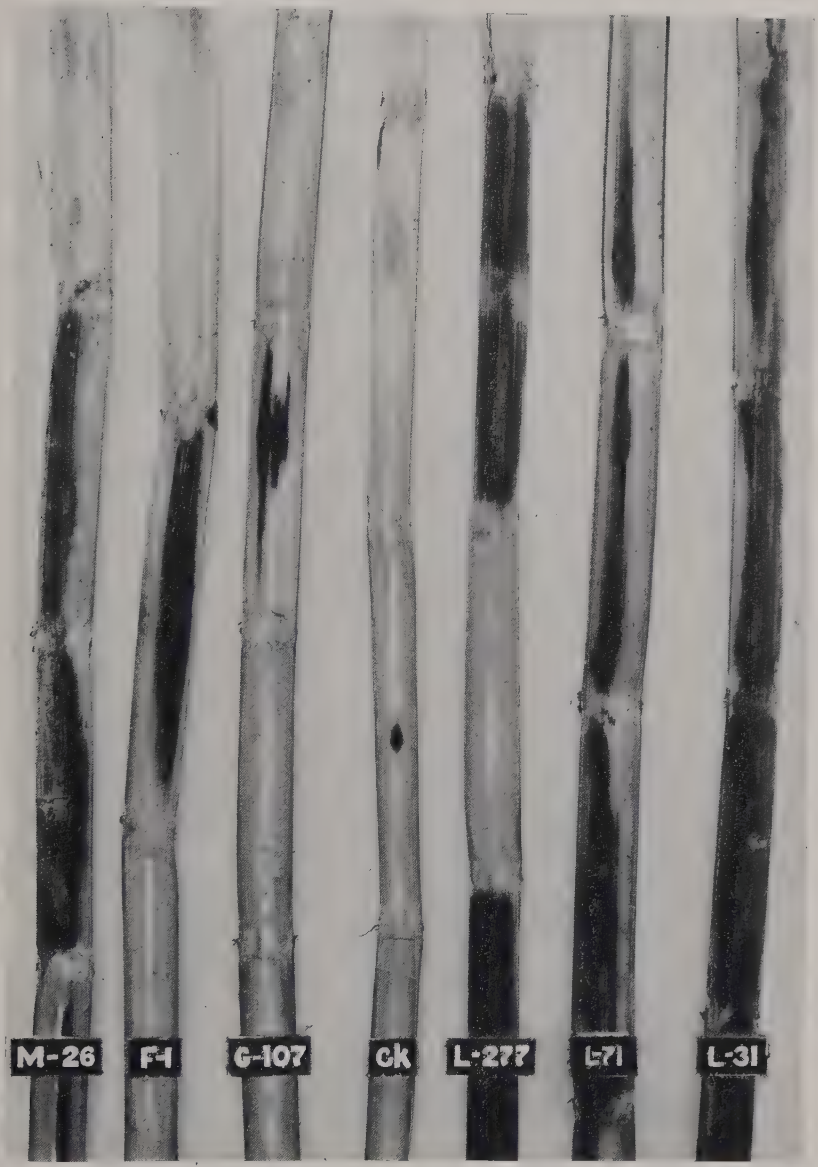
The discovery of an isolate highly virulent toward Co. 281 is naturally of great potential economic significance and also of particular interest in view of an earlier statement by Edgerton (as quoted in the Sugar Bulletin (*3*)) that "Co. 281 is perhaps the most susceptible variety thus far tested." This would indicate that he also was working with an isolate very virulent on this generally resistant variety. If Co. 281 were to be tested only against the isolate L–31, it probably would be classed as a susceptible variety. That L–31 and the isolate tested by Edgerton are chance variants of the general population and not representatives of a possibly specialized subgroup on the ordinarily resistant Co. 281 would appear probable for the reason that no other isolate from stalks of Co. 281 exhibited the same high virulence, a result which has been obtained in all tests involving a total of 38 light-race isolates from stalks of Co. 281 from widely scattered sections of the Sugar Belt that were compared with L–31 on the Co. 281 variety. Therefore, if any extensive specialization of the fungus on this indispensable and generally resistant variety has been taking place, it is inconceivable that more of such variants would not have been found among the fairly widespread collections from the variety.

The results of this test confirm those of tests F and J in showing that relatively high virulence on the particular varieties tested and light color of mycelium of *Colletotrichum falcatum* in culture are correlated characters. In tests F and J, on a susceptible host, this conclusion was limited to the Louisiana red rot flora, but in test K, on a resistant host, the relationship held for the cultures tested from both geographic areas, with the light types from the sirup-producing States (group B) occupying an intermediate position with respect to virulence between the light ones from Louisiana (group A) and the dark types from Louisiana and the sirup-producing States (groups C and D). It is not implied, however, that the above relationships would necessarily hold were the tests conducted on still other genetically different susceptible and resistant hosts.

COMPARATIVE VIRULENCE OF DARK-RACE ISOLATES ON C. P. 807

Test L

As noted in the discussion of the red rot survey (p. 21), the dark cultural races of *Colletotrichum falcatum* appeared to have a special affinity for C. P. 807. Eighty-seven percent of the isolates obtained from this variety during the years 1934–36 were dark types in spite of abundant exposure of the variety to light types. The dark types, furthermore, were isolated exclusively in areas where red rot injury was severe, while light types were obtained at the same time from other varieties in the same locality. From these facts it seemed apparent that the dark types were responsible for the decline of this



Relative virulence of selected isolates of *Colletotrichum falcatum* on the resistant variety Co. 281 in inoculation test K. The highly virulent light-race isolate L–31 is contrasted with four typical light-race isolates from Mississippi (M–26), Florida (F–1), Georgia (G–107), and Louisiana (L–71), and one dark-race isolate (L–277) from Louisiana.

variety, a situation similar to the association of the light types with the failure of P. O. J. 213. It remained to be determined, however, whether the dark isolates obtained from C. P. 807 represented a specialized parasitic race within the morphological group, or whether there was merely a preference of all dark types for the variety. Test L, in which 23 dark-type isolates from C. P. 807 were compared with 18 from other commercial varieties (principally Co. 281, Co. 290, and C. P. 28/19), was planned to throw light on this point.

The 23 cultures from C. P. 807 were obtained in 1935 and 1936 from five plantations in the southeastern parishes and from one each in the northern and western parishes. Those from the southeastern parishes were obtained from fields where marked reductions in germination or stand failures had occurred. Less severe injury to the variety resulted in the northern and western parishes. The 18 cultures from the other commercial varieties represented 6 plantations in the southeastern, 2 in the northern, and 3 in the western parishes. Eight of the isolates were from C. P. 28/19, six from Co. 281, two from Co. 290, and two from P. O. J. 36–M. The results are presented in table 11 and figure 10.

TABLE 11.—*Average virulence index on C. P. 807 of groups of dark-race isolates of Colletotrichum falcatum from that variety in different geographic areas of Louisiana compared with groups from other varieties from the same areas (test L)*

[Virulence index of each isolate based on measurements of 25 lesions in inoculated stalks after 21 days' incubation at 22° to 25° C.]

FROM C. P. 807 (GROUP A)

Group and isolate No.	Source (plantation)	Virulence index	Group difference
			Percent
From southeastern parishes (subgroup A–1):			
L–277	Hollywood	1.43	
L–278	Raceland	1.57	
L–279	Greenwood	1.49	
L–282	do	1.39	
L–283	do	.77	
L–348	Raceland	1.58	
L–349	do	1.19	
L–358	Crescent Farm	1.61	
L–359	do	1.73	
L–364	Reserve	1.92	
L–382	Crescent Farm	.76	
L–394	Raceland	1.94	
L–395	do	1.50	
L–396	do	1.49	
L–397	do	1.99	
L–398	do	1.32	
L–401	Hollywood	1.57	
L–402	do	1.10	
L–403	do	1.05	
Mean of 19 isolates		1.44	
From northern and western parishes (subgroup A–2).			
L–284	Albania	1.32	
L–368	Alma	.92	
L–369	do	1.14	
L–370	do	1.09	
Mean of 4 isolates		1.12	
Percent less virulent than subgroup A–1			22.2
Percent required for $P=0.01$			11.8
Group A, weighted mean		1.39	

TABLE 11.—*Average virulence index on C. P. 807 of groups of dark-race isolates of Colletotrichum falcatum from that variety in different geographic areas of Louisiana compared with groups from other varieties from the same areas (test L)*—Continued

FROM OTHER COMMERCIAL VARIETIES (GROUP B)

Group and isolate No.	Source (plantation)	Virulence index	Group difference
From southeastern parishes (subgroup B-1):			*Percent*
L-312	Crescent Farm	1.16	
L-313	do	.77	
L-346	do	.88	
L-350	Raceland	1.40	
L-363	Reserve	1.74	
L-374	Georgia	1.47	
L-375	do	1.70	
L-376	do	1.95	
L-377	Southdown	1.28	
L-384	Greenwood	1.21	
L-391	do	1.10	
Mean of 11 isolates		1.33	
Percent less virulent than subgroup A-1			7.6
Percent required for $P=0.01$			8.3
From northern and western parishes (subgroup B-2):			
L-267	Cinclare	1.08	
L-270	Alma	1.67	
L-273	Albania	1.94	
L-287	Cinclare	1.48	
L-289	Roy	1.63	
L-291	Cinclare	1.38	
L-325	Glenwild	1.19	
Mean of 7 isolates		1.48	
Percent ± group A-2			+32.1
Group B, weighted mean		1.39	

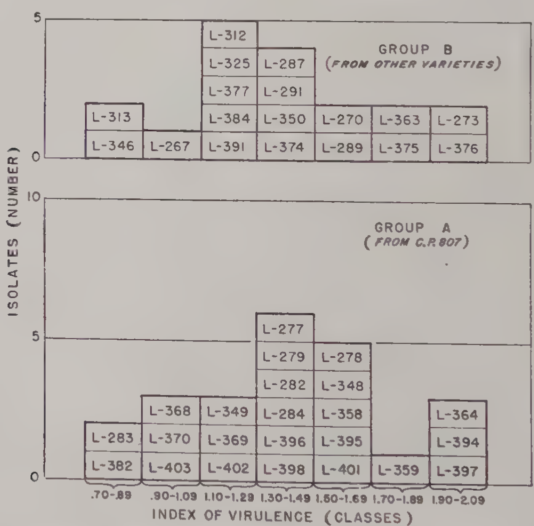


FIGURE 10.—Frequency distribution according to virulence of 41 dark-race isolates of *Colletotrichum falcatum* on C. P. 807 (test L).

The mean virulence index for the 23 isolates from C. P. 807 (1.39) was the same as that for the 18 isolates from the other varieties, and figure 10 reveals a rather even distribution, with respect to virulence, of the C. P. 807 isolates among the general population tested. The

Typical virulence of representative isolates of *Colletotrichum falcatum* indicating specialization of the fungus on the very susceptible variety C. P. 807. Left to right: L–394, representative of a dark-race population from fields of the variety showing practical stand failure; L–346 and L–277, dark-race isolates from fields of C. P. 28/19 and C. P. 807, respectively, which showed no extensive injury; and L–71, typical of the Louisiana light-race flora.

Differing degrees of susceptibility of sugarcane varieties to red rot used for classifying conveniently new seedlings tested by the laboratory method. Left to right: 1, Class 1 (resistant), inoculated internode only partially rotted; 2, class 2 (moderately resistant), inoculated internode rotted but not spreading through the node; 3, class 3 (susceptible), spreading through the nodes; 4, class 4 (very susceptible), practically entire stalk rotted.

fact that there is no piling up of the C. P. 807 forms at the upper end of the virulence scale indicates that these isolates as a group do not constitute a specialized parasitic race. One might assume that the dark isolates from the other varieties would have caused equal injury to C. P. 807 had they come in contact with it.

When all of the isolates in the test are grouped according to the geographic sections of Louisiana from which they were obtained, the average virulence index for the 30 cultures from the southeastern parishes (1.40) (subgroups A–1 and B–1) is not significantly greater than that for the 11 cultures from the northern and western parishes (1.35) (subgroups A–2 and B–2). For the entire morphological group, therefore, there was no correlation between virulence and the geographic distribution of the isolates. But when only the C. P. 807 isolates are considered, such a relationship is indicated. The average virulence index (1.44) for the 19 isolates from the southeastern parishes (subgroup A–1), most of which were obtained from fields where red rot injury to C. P. 807 was severe, was significantly greater (28.6 percent) than the index (1.12) for the four isolates from the northern and western parishes (subgroup A–2), where the disease was less important on this variety. The striking contrast in appearance of inoculated stalks infected with representatives of these two subgroups is shown in plate 9.

The greater red rot injury under field conditions in the southeastern parishes might be explained by the more favorable environmental conditions for the disease prevailing there; i. e. heavier soils with poorer drainage as compared with the lighter soils of the northern and western parishes, but the differences in virulence between the isolates from the two areas when they were tested under identical controlled conditions could not be so explained. The evidence is conclusive that the dark cultural race isolates as a group were associated with the red rot injury to C. P. 807, and this test suggests that the more severe damage in certain areas may have been accentuated by specialized parasitic races within the morphological group. This situation is similar to that in test F for the light cultural races of *Colletotrichum falcatum* in relation to the failure of P. O. J. 213.

DISCUSSION

The data from the virulence-comparison tests definitely establish the fact that isolates of *Colletotrichum falcatum* differ in virulence as measured by their rate of spread in both a resistant and a susceptible host, confirming the author's previous preliminary evidence in this respect (*10*). To a certain degree these differences on all varieties thus far tested are associated with differing morphological characters, the isolates of the light cultural race, in general, being more virulent than those of the dark. This is particularly true of the Louisiana flora. There was evidence also that certain groups of both light and dark-race isolates show a degree of physiologic specialization. This was true with respect to the light-race isolates from P. O. J. 213 in test F, the dark-race isolates from C. P. 807 in test L, and the single light-race isolate L–31 in test K. The differences between the isolates or groups of isolates in these tests were based on their rate of spread within the host tissues, and do not represent the high degree of specialization demonstrated in the rust fungi, in which the differentia-

tion of physiologic forms is based in part on immunity of certain hosts toward certain parasitic races of the fungus.

The results are somewhat contradictory to the findings of Tims and Edgerton (*67, p. 4*), who state:

While these strains have varied in their ability to produce spores and in the type of growth, consistent physiological differences have not been noted. In inoculation experiments using resistant and susceptible varieties, considerable variations have been noticed which suggest a degree of specialization but striking examples of this have not been demonstrated.

Relation of Specialized Races of Colletotrichum falcatum to the Failure of P. O. J. 213

One of the primary objects of the investigation was to determine the cause of the sudden decline of P. O. J. 213, a variety that had been considered highly resistant to red rot when released. So far as the Louisiana industry is concerned a discussion of its failure is now largely academic. Nevertheless, the variety is annually assuming increasing importance in the sirup-producing areas of the Southeastern States, where prior to 1938 it had shown no more indication of declining from red rot than it did in the early years of its cultivation in Louisiana, and an understanding of the cause of its failure in one area should be of value in preventing similar losses to the industry in another section. Furthermore, such information would be of great importance in predicting the performance of new seedling canes prior to their release, thereby obviating the serious losses sustained by the industry when an important variety fails. If sugarcane growers are to be freed of the constant threat of the failure of any one of the commercial varieties on which the industry depends, from causes that can neither be predicted nor controlled, it is of vital importance to them that the failures which have occurred in the past should be explained.

In view of the absence of cultures of *Colletotrichum falcatum* from the period prior to the release of P. O. J. 213, when Louisiana Purple and D–74 were the principal commercial varieties of Louisiana, or from the period of its successful commercial cultivation (1926–29), to compare with those obtained since 1930, it is impossible to prove definitely that the sudden decline of the variety was due to a change in the predominating red rot flora. The data presented in this bulletin, however, offer circumstantial evidence that such was the case. Such a conclusion is supported by the following facts:

(1) The dominance of the light cultural race of *Colletotrichum falcatum* from 1934–37 on P. O. J. 213, when in many cases it was more abundantly exposed to the dark cultural race, which had multiplied on other varieties in surrounding fields. This suggests a definite affinity of the former for this variety.

(2) Proof that these light-race isolates as a group are relatively more virulent on P. O. J. 213 than the dark-race isolates.

(3) The successful cultivation of the variety with no indication of red rot injury, at least until 1938, in the sirup-producing States, where it had been exposed to the dark race to the practical exclusion of the light. That this apparent resistance is not due to escape from exposure to the fungus, because of the absence of the moth borer, may

be judged from the fact that borer-free P. O. J. 213 cane banked in Louisiana is invariably severely rotted as the result of penetration by Louisiana forms of the fungus through the root primordia and into the internodes; and that it is not due to differences in soil, climate, and other environmental factors between Louisiana and the sirup States that might have an influence on red rot may be assumed, since such differences have not operated to prevent severe red rot injury to Cayana and Louisiana Purple in the sirup areas.

(4) Red rot inoculation tests with P. O. J. 213 from all parts of the cane-growing areas of the South, from different soil types, and with different fertilizer treatment, which have shown that under controlled laboratory conditions the inherent susceptibility of the variety is a constant character.

In addition to these established facts relative to P. O. J. 213 is the evidence indicating the association of certain groups of isolates of *Colletotrichum falcatum* with C. P. 807, making more plausible the explanation of the failure of P. O. J. 213 on the basis of specialized races of the fungus.

VARIETAL RESISTANCE AND SUSCEPTIBILITY

In the Department's coordinated sugarcane-breeding and disease-testing program obtaining varieties resistant to red rot is a major objective, and constitutes a basis for initial seedling selection. For, unless varieties are selected that possess some degree of resistance to the disease, they would be dangerous to recommend to growers. The surprising rarity of satisfactory resistance necessitates this emphasis on red rot resistance, which, as pointed out later under the discussion of the control of red rot, must be the main dependence in the prevention of serious annual losses or the periodic outbreak of epidemics.

In order that the resistance or susceptibility of the thousands of seedlings produced at the Canal Point station each year may be determined before they are selected and subjected to further agronomic, pathological, physiological, and chemical studies, it has been necessary to devise methods of testing them for their reaction to red rot as well as to other diseases. With respect to mosaic resistance, which is also a major consideration in the initial seedling selection work, information may usually be obtained from the results of inoculations in the field the first year. With root rot (*54*) and red rot, however, as mentioned earlier in this bulletin (p. 45), the influence of weather and soil conditions on the severity of the diseases in the field is such that a susceptible variety may escape serious injury in some years and thus appear to be resistant. This is particularly true with respect to germination injury to seed cane. Depending solely on field observations, therefore, would frequently require several years before the reaction of a variety to red rot could be evaluated correctly. Furthermore, it is physically impossible to test adequately any large number of seedlings in the field in any one year. Consequently, what may be termed the laboratory method has been employed as a means of providing information as to the inherent resistance or susceptibility of large numbers of seedlings the first year after they are produced.

METHODS OF TESTING

The laboratory method consists of determining the rate of spread of the red rot fungus in inoculated cuttings of mature stalks of cane under conditions favorable for the development of the disease, as compared with that in similarly treated cuttings of standard varieties, the reaction of which in the field and in the laboratory is known. The procedure is essentially the same as that already described for the virulence trials. For testing the seedlings, however, the cuttings are not surface-sterilized and "field-run" cane is used without regard to borer injury. Elimination of natural infection is no object, since the purpose of these tests is to determine the susceptibility of the variety under optimum conditions for the development of red rot. After inoculation, the canes are incubated in sawdust bins or in insulated rooms, as described for the virulence trials, and the red rot ratings are made on the basis of visual examination of the split stalks in comparison with similarly treated cuttings of standard varieties, the red rot reaction of which is known.

The laboratory comparisons are supplemented by field trials of the advanced agronomic selections, particularly those in red rot classes 3 and 4. The general procedure is to inoculate 100-eye bundles of cuttings and to plant in mixed or heavy soil to favor red rot. In one lot the inoculum is introduced into holes punched in the stalks, and in another it is smeared over the nodes (for class-5 ratings). An uninoculated control of each variety is also included. From three to five replications are used if sufficient seed cane is available. The plantings are generally made in October, and the effect of red rot on germination is determined the following spring.

CLASSES OF RESISTANCE

In the beginning of the testing work only four classes of red rot resistance and susceptibility were used (*11*), but as the great importance of resistance to nodal infection in determining the extent of red rot injury to seed cane became apparent, it seemed advisable to subdivide class 4, grouping those canes that are susceptible to nodal infection as well as to rapid spread in the tissues in class 5. Split, infected stalks illustrating the first four classes are shown in plate 10. The definition of the five classes is as follows:

Class 1. Resistant to nodal infection and to spread in the tissues.

Class 2. Moderately resistant to spread in the tissues; resistant to nodal infection.

Class 3. Susceptible to spread in the tissues, but characterized by temporary check of the fungus at the nodes; resistant to nodal infection under most conditions.

Class 4. Very susceptible to spread in the tissues with little or no checking of the fungus at the nodes; resistant to nodal infection under most conditions.

Class 5. Very susceptible to spread in the tissues and to nodal infection.

There are, of course, no sharply defined limits between the classes with the possible exception of 4 and 5, and it should not be assumed that different varieties grouped in any one class are necessarily of equal susceptibility to the disease. Nevertheless, such a classification is convenient in evaluating the commercial possibilities of seed-

lings, and in general indicates the relative degree of red rot injury, particularly to seed cane, that may be expected in average years.

Field injury from red rot is influenced by many factors, such as the vigor of the variety, susceptibility to borer attack, susceptibility to root rot, the type of soil in which the cane is planted, drainage, the preparation of the seedbed, as well as weather conditions. The relation of these factors to germination and red rot injury to seed cane have been discussed by the author (*13*) elsewhere. These are not taken into account in determining inherent resistance or susceptibility to the spread of the disease in the tissues, but they must be considered in evaluating the commercial possibilities of a variety. C. P. 807, for example, is very susceptible to red rot, yet its great vigor and resistance to root rot have frequently enabled it to escape the losses that would have been sustained by an equally susceptible but less vigorous cane. This variety may suffer a severe stand reduction in the spring, and still produce a fairly satisfactory yield of cane in the fall because of its vigor and root rot resistance.

C. P. 28/19, on the other hand, which is moderately resistant to red rot but which Rands (*54*) considers susceptible to root rot, in some years may be more severely injured by red rot than other varieties that are more susceptible to red rot but resistant to root rot, as brought out in the discussion of environmental conditions (p. 45). Rands has described the extreme root rot damage that frequently occurs to seed roots of this variety as they emerge, thus arresting the normal physiological activity of the cutting and favoring the development of red rot and other seed-cane diseases. From extensive field examinations of seed cane of C. P. 28/19 during the winter and spring it seems this accounts for much of the seed deterioration that has occurred in this variety during the seasons of 1935 and 1936, rather than inherent susceptibility to red rot.

Susceptibility to borer attack is of importance since it largely determines the extent to which many varieties are subject to red rot infection. Ellisor and Jaynes[12] have found that Co. 290 (susceptible to red rot) shows a relatively light borer infestation, and being resistant to nodal infection, red rot injury to mill cane is consequently light. C. P. 29/116, on the other hand, is very subject to borer attack, and ordinarily mill cane of this variety is much more severely affected by red rot than that of Co. 290.

The red rot resistance of the growing plant possessed by some varieties, notably C. P. 807, is also important. Red rot spreads relatively little in vigorously growing C. P. 807, whereas in the semidormant seed cuttings, spread is very rapid. C. P. 29/99 (class 4), and 29/116 (class 3), do not have this growing-plant resistance to any marked degree, red rot frequently spreading through several joints of the growing stalk from a single point of infection.

It is obvious then, that any attempt to classify sugarcane varieties according to red rot resistance must be arbitrary to a certain extent. However, some basis of classification is essential in the pathological testing of seedlings in the Department's breeding program. Because of the great importance of red rot as a seed-cane disease, this point is

[12] ELLISOR, L. O., and JAYNES, H. A. SUSCEPTIBILITY OF VARIOUS VARIETIES OF SUGARCANE TO INFESTATION BY THE SUGARCANE BORER. Paper read at a conference of entomologists of the State of Louisiana held at Tallulah, La., May 7–8, 1937. (Unpublished.)

particularly stressed, and the classes of resistance have been defined with special reference to this type of injury.

RELATION OF RACES OF THE CAUSAL FUNGUS TO SEEDLING TESTING

Proof of the existence of both morphologic and parasitic races of *Colletotrichum falcatum* complicates the matter of adequately testing for red rot resistance the hundreds of seedlings selected each year in the Department's breeding program. It is possible that if a variety were tested with only one isolate of the fungus or representatives of one morphologic race, it would be classed as resistant, whereas it might be susceptible to others. The susceptibility of the generally resistant variety Co. 281 to the isolate L–31 (see test K) illustrates this possibility. As a rule, however, varieties classed as resistant or susceptible to one isolate have not had their classification changed when tested with larger numbers of isolates.

In view of the physical impossibility of testing several hundred seedlings each year with a large number of isolates of *C. falcatum*, the procedure has been to inoculate five stalks of each of the field selections with one light-race isolate of *C. falcatum*. A light race is used since the comparative virulence tests showed that in general this morphologic group was relatively more virulent than the dark races; i. e., spread through the sugarcane stalk was faster within a given incubation period. Field selections giving a class-4 reaction are discarded, except those that the chemical analyses have shown are high in sucrose. A seedling handicapped by high susceptibility to red rot (or other major diseases) and low or only fair sucrose is not considered commercially promising, even though it may have some other desirable qualities. Susceptible seedlings of high sucrose are then planted in a special red rot nursery on heavy soil where the influence of the disease on the variety under field conditions may be further studied. Seedlings that give poor stands, either as plant cane or stubble, are discarded, but those (like C. P. 807) that appear to possess considerable field resistance to red rot, may be assigned C(anal) P(oint) numbers and turned over to the agronomists for field trials.

After the preliminary red rot rating on the field selections described on page 70 further testing for red rot is usually reserved for the reduced number of seedlings that, 2 or 3 years later, are selected by the agronomists as having greatest commercial promise. These, perhaps 5 to 10 in number each year, are inoculated with several isolates of both morphologic races of *Colletotrichum falcatum*, and these tests are supplemented by observations on their red rot reaction in the field on both light and heavy soil types. Varieties that survive these tests without showing important germination injury or stubble deterioration by red rot are considered safe for commercial release, so far as this disease is concerned.

Even after a variety has thus been tested and rated commercially resistant to the disease, there is no assurance that some race of *C. falcatum* particularly virulent toward it may not multiply on it following its release. Since the beginning of the present testing procedure this has not occurred, but that it is a potential danger is illustrated by the discovery of an isolate virulent toward Co. 281. There is always the possibility that some combination of environmental conditions may favor the increase of such a strain on an ordinarily resistant

variety. This necessitates annual inspection of representative plantation fields of the commercial varieties, for it is only by such constant vigilance that the building up of a specialized red rot population on any variety can be detected.

REACTION OF SACCHARUM SPECIES AND OF INTERSPECIFIC AND MISCELLANEOUS HYBRIDS

Aside from the immediate practical consideration of obtaining disease-resistant commercial types, an important aim of the disease-testing program has been the determination of the original source or sources of resistance to red rot in order that greater utilization of resistant varieties or species might be made in the breeding program. Thus far a partial survey of the principal species and varieties of the genus *Saccharum* has been undertaken.

Varieties representing the five generally accepted species of *Saccharum* and a number of interspecific and miscellaneous hybrids are listed below with their red rot ratings, as determined by the laboratory method. It will be noted that 32 of the varieties of *S. officinarum* L. (group I) listed were very susceptible (class 4), 13 of which are natural varieties. Four were susceptible (class 3), and it seems significant that they are not naturally occurring noble varieties, but hybrids of unknown origin. It is possible that their slightly lesser susceptibility to red rot is the result of mixture of noble canes with a variety of a more resistant group. The conclusion seems justified, therefore, that high susceptibility to red rot is characteristic of this species, a view which is further attested by the numerous references in the literature to the red rot injury suffered by the noble canes in commercial cultivation.

Most of the varieties of the New Guinea collection of Brandes and Jeswiet, tentatively classed as *Saccharum officinarum*, were typical of the noble canes in their red rot reaction, being very susceptible. However, three were susceptible, one moderately resistant, and one resistant. Presumably, this might indicate that the latter ones are of hybrid origin.

Group I:

Species and variety:	*Red rot rating*
Noble canes (*Saccharum officinarum* L.):	
B. 208	Susceptible.
B. 726	
Ba. 6032	Very susceptible.
Ba. 6835	
Ba. 11569	Susceptible.
Badila	
Batjan	
BH 10/12	
Crystalina	
D–74	
D–95	
D–109	
Diamond 10	Very susceptible.
E. K. 2	
Fiji	
Hak-kwat-che	
H. 28–5009	
H. 28 5019	
H. Q. 5	
H. Q. 409	

	Red rot rating
Group I—Continued.	
Species and variety—Continued.	
Noble canes (*Saccharum officinarum* L.)—Con.	
L–511	Very susceptible.
Loethers	Very susceptible.
Louisiana Purple	Very susceptible.
Louisiana Striped	Very susceptible.
Mahona	Very susceptible.
Otaheite	Very susceptible.
P. O. J. 100	Very susceptible.
Q. 813	Very susceptible.
S. C. 12/4	Very susceptible.
Simpson	Very susceptible.
S. J. 4	Susceptible.
S. W. 3	Very susceptible.
S. W. 111	Very susceptible.
S. W. 499	Susceptible.
Yellow Caledonia	Very susceptible.
Wit Ceram	Very susceptible.
New Guinea collection of Brandes and Jeswiet:[1]	
28 numbers	Very susceptible.
N. G. 106–408	Susceptible.
N. G. 106–426	Susceptible.
14 N. G. 124	Moderately resistant.
14 N. G. 241	Susceptible.
14 N. G. 190	Resistant.
Group II:	
Indian canes (*S. barberi* Jeswiet):	
Chunnee	Very susceptible.
Hatooni	Moderately resistant.
Group III:	
Chinese canes (*S. sinense* Roxb.):	
Cayana	Very susceptible.
Chukche	Susceptible.
Khera	Susceptible.
Kinar	Susceptible.
Oshima	Susceptible.
Merthi	Very susceptible.
Uba	Susceptible.
Tekcha	Susceptible.
Yontanzan	Susceptible.
Group IV:	
Wild canes (*S. robustum*) (?):	
N. G. 251	Susceptible.
Group V:	
Wild canes (*S. spontaneum* L.):	
Indian forms:	
Assam (Gehra Bon) (Imp. No. 618)	Moderately resistant.
Coimbatore (Imp. No. 238)	Moderately resistant.
Coimbatore (Imp. No. 470)	Moderately resistant.
East Bengal (Imp. No. 230)	Moderately resistant.
Godavari (Rella Gaddi) (Imp. No. 617)	Moderately resistant.
East Indian forms:	
Glagah Djatiroto (Imp. No. 569)	Moderately resistant.
Glagah Kepandjen (Imp. No. 558)	Moderately resistant.
Glagah Kloet (Imp. No. 564)	Moderately resistant.
Glagah Pasoeroean (Imp. No. 555)	Moderately resistant.
Glagah Tabongo (Imp. No. 578)	Moderately resistant.
Formosan forms:	
3 numbers	Moderately resistant.
1 number	Very susceptible.

[1] Tentatively included in *Saccharum officinarum*.

<table>
<tr><td>Group VI:</td><td></td></tr>
<tr><td> Interspecific hybrids:</td><td></td></tr>
<tr><td>   (a) I × V—</td><td>Red rot rating</td></tr>
<tr><td>   Hind's Special</td><td rowspan="4">Very susceptible.</td></tr>
<tr><td>   I. 1081</td></tr>
<tr><td>   Kassoer</td></tr>
<tr><td>   Toledo</td></tr>
<tr><td>   (b) I × II—</td><td></td></tr>
<tr><td>   P. O. J. 36</td><td>Susceptible.</td></tr>
<tr><td>   P. O. J. 213</td><td>Very susceptible.</td></tr>
<tr><td>   P. O. J. 234</td><td>Resistant.</td></tr>
<tr><td>   (c) I × III—</td><td></td></tr>
<tr><td>   C. H. 64–21</td><td>Very susceptible.</td></tr>
<tr><td>Group VII:</td><td></td></tr>
<tr><td> Miscellaneous hybrids (parent and commercial varieties):</td><td></td></tr>
<tr><td>   C. P. 807</td><td>Very susceptible.</td></tr>
<tr><td>   C. P. 1161</td><td>Resistant.</td></tr>
<tr><td>   C. P. 1165</td><td rowspan="2">Susceptible.</td></tr>
<tr><td>   C. P. 27/139</td></tr>
<tr><td>   C. P. 28/11</td><td>Resistant.</td></tr>
<tr><td>   C. P. 28/19</td><td>Moderately resistant.</td></tr>
<tr><td>   C. P. 28/36</td><td rowspan="2">Resistant.</td></tr>
<tr><td>   C. P. 28/122</td></tr>
<tr><td>   C. P. 29/116</td><td>Susceptible.</td></tr>
<tr><td>   C. P. 29/284</td><td>Resistant.</td></tr>
<tr><td>   C. P. 29/320</td><td>Susceptible.</td></tr>
<tr><td>   Co. 213</td><td>Very susceptible.</td></tr>
<tr><td>   Co. 281</td><td>Resistant.</td></tr>
<tr><td>   Co. 290</td><td>Susceptible.</td></tr>
<tr><td>   P. O. J. 2222</td><td rowspan="5">Very susceptible.</td></tr>
<tr><td>   P. O. J. 2354</td></tr>
<tr><td>   P. O. J. 2364</td></tr>
<tr><td>   P. O. J. 2714</td></tr>
<tr><td>   P. O. J. 2725</td></tr>
<tr><td>   P. O. J. 2727</td><td rowspan="2">Susceptible.</td></tr>
<tr><td>   P. O. J. 2775</td></tr>
<tr><td>   P. O. J. 2822</td><td>Very susceptible.</td></tr>
<tr><td>   P. O. J. 2878</td><td>Moderately resistant.</td></tr>
<tr><td>   P. O. J. 2883</td><td>Susceptible.</td></tr>
<tr><td>   U. S. 785</td><td rowspan="4">Very susceptible.</td></tr>
<tr><td>   U. S. 1078</td></tr>
<tr><td>   U. S. 1643</td></tr>
<tr><td>   U. S. 1694</td></tr>
<tr><td>   Uba Marot</td><td>Susceptible.</td></tr>
</table>

Unfortunately, only two varieties of *Saccharum barberi* (group II) were available for study, one of which (Chunnee) was classed as very susceptible, and one (Hatooni) as moderately resistant. In view of Barber's (*22*) and Butler and Hafiz Kahn's (*30*) statements that the Indian thin canes (*S. barberi*) were on the whole more resistant to red rot than the thick (*S. officinarum*), one might expect to encounter more resistant varieties in this group if larger numbers were studied. On the other hand, the fact that these authors considered Chunnee to be resistant, in contrast to the high susceptibility shown by it to the Louisiana forms of the red rot fungus in the present study, lessens the probability of finding other varieties in this botanical group resistant to the forms of the fungus present in the United States. Apparently these or certain ones of them at least are more virulent than the Indian forms.

The Chinese canes (*S. sinense*) (group III), on the whole, were less susceptible than the noble canes, only two out of nine being classed as very susceptible. The one representative of *S. robustum* was also in the susceptible class.

Fourteen varieties, or collections, of *S. spontaneum* were available for testing, 13 of which were classed as moderately resistant and 1 as very susceptible. The large pith cavities in the stalks of *S. spontaneum* make it difficult to obtain typical red rot symptoms comparable to those in the solid stalk canes, the typical mottling generally being absent. In only 1 of the 14 forms of *S. spontaneum* tested did the fungus spread through the nodes from the inoculated internodes.

Four hybrids of *S. officinarum* × *S. spontaneum* (group VI) and one of *S. officinarum* × *S. sinense* (group VI–c) were available for study, all of which were very susceptible, as would be expected because of the high susceptibility of the parents.

Of the three hybrids of *Saccharum officinarum* × *S. barberi* (group VI–b) P. O. J. 234 was resistant, P. O. J. 36 susceptible, and P. O. J. 213 very susceptible. The parents of the P. O. J. 36 (Louisiana Striped × Chunnee) and P. O. J. 234 (Louisiana Purple × Chunnee) are all very susceptible. Whether one or both of the parents supplied the resistance factor can only be speculated upon, but in view of the high susceptibility of all of the noble canes tested, and the statements of Barber (*22*) and Butler and Hafiz Khan (*30*) regarding the relative susceptibility of *S. officinarum* and *S. barberi*, one might assume that resistance would be more apt to occur in a variety of the latter rather than of the former.

The miscellaneous hybrids in group VII represent a selected group of commercial and parent canes, constituting only a partial list of the several hundreds of hybrid seedlings that have been tested for red rot. It will be noted that only six of these are resistant to the disease; namely, Co. 281 and the C. P. numbers 1161, 28/11, 28/36, 28/122, and 29/284. Of these six, 28/11, 28/36, and 29/284 are seedlings of Co. 281, and presumably owe their red rot resistance to it. That only six red rot-resistant varieties should occur among the more than 100 parent canes tested that have been used in the breeding work at the Canal Point station emphasizes the rarity of resistance to the disease in sugarcane varieties and the difficulty of obtaining seedlings combining resistance with the other qualities essential in a commercial cane.

The origin of resistance in Co. 281 might conceivably be traced to two sources: to *Saccharum barberi* through its female parent, P. O. J. 213; and to *S. spontaneum* through its male parent Co. 206 (a seedling of Ashy Mauritius × *S. spontaneum* (*71*)). A reason for suspecting that some forms of *S. spontaneum* may be resistant, or possess resistance as a recessive factor, is the fact that all of the resistant seedlings that have been encountered among the thousands produced at the Canal Point station, carry at least a trace of *S. spontaneum* inheritance; and while some of them are also traceable to *S. barberi*, many are not.

The C. P. 1161, so far as the parentage is known, is a descendant of P. O. J. 213 (very susceptible), and thus carries the inherited characters of both *Saccharum officinarum* and *S. barberi*. It is believed, however, that *S. spontaneum* may have entered its lineage through U. S. 1484, of which it is a seedling. U. S. 1484 is presumably a selfed

seedling of P. O. J. 213 but shows certain wild cane characteristics, which make it appear probable that a form of *S. spontaneum* may have been its male parent.

The C. P. 29/122 is predominantly of *S. officinarum* parentage, but carries a trace of *S. spontaneum* through Kassoer, and of *S. barberi* through P. O. J. 213.

PROGENY TESTS

Information as to the distribution of resistance to red rot and other diseases among seedlings of the most valuable crosses is an aid to the cane breeder in the choice of parent varieties. The tests for red rot resistance require a considerable amount of time and labor, because of which, as well as the lack of facilities for maintaining controlled temperatures for incubating the inoculated cane, only a few complete seedling progenies have been tested thus far. Four crosses have been studied: three that have given large numbers of promising selections, and are thus considered most valuable commercially; and a fourth, because it represents a backcross of a very susceptible seedling of Co. 281 on its resistant parent. The results are presented in table 12.

Crosses 1 and 2 (Co. 281 × U. S. 1694 and Co. 281 × P. O. J. 2878) in table 12 were studied in considerable detail. These crosses are of interest because of the high quality of many of their seedlings and the wide divergence of the parent canes' reactions to various diseases. Co. 281, for example, is resistant to red rot and susceptible to mosaic, while U. S. 1694 is very susceptible to red rot and resistant or immune to mosaic. P. O. J. 2878 is moderately resistant to red rot and resistant to mosaic.

TABLE 12.—*Number and percentage of seedlings from four complete progenies falling in four classes of red rot resistance or susceptibility*

Progeny No.	Cross and red rot rating of parents[1]	Class 1 (resistant)		Class 2 (moderately resistant)		Class 3 (susceptible)		Class 4 (very susceptible)		Total
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*
1	Co. 281 (1) × U. S. 1694 (4) (1931 series).	17	4.4	102	26.2	178	45.6	93	23.8	390
2	Co. 281 (1) × P. O. J. 2878 (2) (1931 series).	68	21.5	79	25.0	118	37.3	51	16.1	316
3	P. O. J. 2725 (4) × C. P. 1161 (1) (1932 series).	17	16.2	15	14.3	28	26.7	45	42.9	105
4	Co. 281 (1) × C. P. 30/23[2] (4) (1932 series).	9	8.9	16	15.8	20	19.8	56	55.4	101

[1] Numbers in parentheses indicate the red rot rating of the parent variety.
[2] C. P. 30/23 is a seedling of Co. 281 × U. S. 1694.

Of the seedlings produced from these crosses in the 1931 Canal Point series, 390 from the Co. 281 × U. S. 1694 and 316 from the Co. 281 × P. O. J. 2878 crosses were tested for red rot. These represented all of the seedlings from the crosses that produced sufficient mature cane to provide a three- to five-stalk sample for a red rot test. All of the seedlings had been inoculated with mosaic, and some of them were so severely stunted by this disease that they did not produce any mature stalks. While this resulted in the elimination of some seedlings from the red rot tests, it is believed that the numbers tested were large enough to give a fairly reliable indication of the distribution of red rot resistance in the progenies.

Crossing the resistant female, Co. 281, with the moderately resistant male, P. O. J. 2878 (cross 2, table 12), gave, as would be expected, a considerably higher percentage of resistant seedlings (classes 1 and 2) than crossing the same female with the very susceptible male, U. S. 1694 (cross 1). The percentage of very susceptible or commercially unsuitable seedlings was correspondingly higher in cross 1 than in cross 2. From these figures it is apparent that crossing the very valuable female Co. 281 with P. O. J. 2878 may be expected to yield a higher percentage of red rot-resistant seedlings than with U. S. 1694. The distribution of resistance among the field selections from the two crosses will be considered later.

In cross No. 3 (table 12), the female parent, P. O. J. 2725, is very susceptible, and the male parent, C. P. 1161, is resistant. This cross is of particular interest, because many of the seedlings combine the large barrel, ease of shucking, and other desirable harvesting qualities inherited from P. O. J. 2725, with the vigor of C. P. 1161. Unfortunately, however, many of them are below the necessary standards in sucrose.

It will be noted from table 12 that the percentage of seedlings in the two resistant classes is the same in this cross as in cross 1, where a resistant female was crossed on a very susceptible male, although the percentage of highly resistant seedlings (class 1) in cross 3 was nearly four times as great as in cross 1. Conversely, the number of very susceptible seedlings (class 4), i. e., those unsuited for commercial utilization, was much greater in cross 3 than in cross 1. Thus, so far as these crosses may be judged by the numbers of seedlings tested, it seems that cross 1 may be expected to give seedlings of generally higher quality with respect to red rot resistance than cross 3.

In cross 4, which represents a backcross of a very susceptible seedling of Co. 281 × U. S. 1694 (C. P. 30/23) on its resistant parent (Co. 281), there was apparently no increase in the percentage of resistant seedlings considering the cross as a whole. The percentage of highly resistant seedlings (class 1) was greater in the backcross, but the number of very susceptible, or commercially unsuitable canes, was more than double that in cross 1. This suggests that susceptibility is a partially dominant character and that bringing together of recessives (resistant factors) resulted in a double-recessive or dominance of resistance in the case of the highly resistant seedlings.

DISTRIBUTION OF RESISTANCE AMONG THE SELECTIONS FROM TWO COMPLETE PROGENIES OF COMPLEX INHERITANCE

Each of the seedlings in crosses 1 and 2 had also been rated with respect to its growth habit, its agronomic promise, and analyzed for sucrose (except those that did not produce mature stalks). With these data available, an attempt was made to determine whether resistance or susceptibility to red rot was correlated with one or more of the other measurable characters. A previous analysis of the data with respect to red rot and mosaic had indicated that there was no relationship between resistance and susceptibility to these diseases in these progenies (*56*).

Growth Habit of Co. 281

It would be of considerable practical importance in making selections in the field for commercial type if a relationship could be established between red rot resistance and some easily recognized character,

such as growth habit. Since a considerable number of the seedlings of Co. 281 inherit its characteristic growth habit, knowledge of the existence of any correlation between this character and red rot resistance would be of value.

In table 13 the red rot classification of the seedlings of the Co. 281 × P. O. J. 2878 cross, which were classed as approximating the Co. 281 type of growth habit,[13] are compared with the entire progeny. It will be noted that the percentage of resistant and susceptible seedlings is nearly the same for the entire progeny and for the 63 rated as approximating the Co. 281 type of growth, indicating that for this cross there is no relation between the red rot resistance of Co. 281 and its growth habit.

TABLE 13.—*Number of seedlings from the cross Co. 281 × P. O. J. 2878 in four classes of red rot susceptibility grouped according to habit of growth*

Red rot class	With growth habit of Co. 281		Entire progeny	
	Number	*Percent*	*Number*	*Percent*
Resistant	11	17.6	68	21.5
Moderately resistant	15	23.8	79	25.0
Susceptible	23	36.5	118	37.3
Very susceptible	14	22.2	51	16.1

AGRONOMIC CHARACTER OF SEEDLINGS

In table 14 the complete progenies and the agronomic field selections from them, i. e., those seedlings selected for good vigor, desirable growth habit, and other characters desired in a commercial variety, are classified according to their red rot reaction. The sucrose analyses were not taken into account in making these selections.

In the Co. 281 × U. S. 1694 cross the selections were definitely more susceptible than the entire progeny, the percentage of selected seedlings in the two commercially resistant classes being less than half of those in the complete progeny. For the Co. 281 × P. O. J. 2878 cross the percentages of commercially resistant (classes 1 and 2) and susceptible (classes 3 and 4) seedlings were practically the same for both the complete progeny and the selections. Data from additional samples of the progenies of these and other crosses would be necessary to permit any generalization as to correlation between red rot resistance and agronomic characters.

TABLE 14.—*Number of seedlings in four classes of red rot susceptibility for the entire progenies and for the agronomic selections from the progenies of the crosses Co. 281 × U. S. 1694 and Co. 281 × P. O. J. 2878*

[1931 series]

Progeny	Total seedlings	Class 1 (resistant)		Class 2 (moderately resistant)		Class 3 (susceptible)		Class 4 (very susceptible)	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Co. 281 × U. S. 1694 entire progeny	390	17	4.4	102	26.2	178	45.6	93	23.8
Agronomic selections only	75	0	0	11	14.7	44	58.7	20	26.7
Co. 281 × P. O. J. 2878 entire progeny	316	68	21.5	79	25.0	118	37.3	51	16.1
Agronomic selections only	93	15	16.1	28	30.1	33	35.5	17	18.3

[13] Data on growth habit furnished by G. B. Sartoris.

Sucrose Content of the Juice

Table 15 shows the number of seedlings from the two progenies and the number of seedlings falling in each class of red rot susceptibility, with their average sucrose analyses.

Table 15.—*Number of seedlings and average sucrose analyses of two complete progenies and their agronomic selections arranged according to classes of red rot susceptibility*

Progeny	Class 1 (resistant)		Class 2 (moderately resistant)		Class 3 (susceptible)		Class 4 (very susceptible)		Total	
									Seedlings	Weighted average sucrose
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Co. 281 × U. S. 1694 progeny	16	10.32	103	10.14	176	10.20	92	10.01	387	10.14
Co. 281 × U. S. 1694 selections	0	-------	12	10.83	45	10.75	17	10.95	74	10.81
Co. 281 × P. O. J. 2878 progeny	67	10.15	81	9.98	116	8.95	48	9.68	312	9.59
Co. 281 × P. O. J. 2878 selections	15	10.52	30	10.49	33	9.92	17	9.33	95	10.09

From these figures it is apparent that for the Co. 281 × U. S. 1694 cross there is no relation between red rot susceptibility and sucrose for the progeny as a whole or for the agronomic selections, the average sucrose analyses for each of the four groups being practically identical.

In the Co. 281 × P. O. J. 2878 cross there was a tendency for the low-analyses canes to fall in the susceptible classes, a trend that is very consistently followed when the agronomic selections are considered alone. Although it is of interest to note this trend, the differences in analyses between classes are not significant. It is possible, of course, that the apparent tendency of the very susceptible canes to fall in the low-analysis group is due to greater borer and red rot injury to the standing cane of the seedlings in these classes, with a consequent reduction in sucrose.

DISTRIBUTION OF RESISTANCE AMONG SELECTIONS FROM COMMERCIAL CROSSES

In the routine seedling-testing procedure at the Houma station, as outlined previously (*14*, *15*, *56*) the initial nursery selections, termed field selections, are made by the pathologists. These are made on the basis of freedom from, or apparently slight injury by, mosaic and other diseases, root rot resistance, vigor, erectness, stalk size, ease of shucking, and general appearance desired in a commercial cane. The field selections made from several of the hundreds of crosses tested during the past 7 years have been such as to gain some idea of the value of the crosses. These are listed by series in table 16. While the numbers here represented are probably too small to give any conclusive evidence as to the value of the crosses with respect to red rot resistance, in certain cases it is believed that the comparatively large numbers of red rot resistant seedlings have not resulted purely by chance. It seems reasonable to conclude, therefore, that crosses 1, 6, and 22 (Co. 281 × P. O. J. 2878); 2, 7, 18, and 23 (Co. 281 × U. S. 1694);

8 (Co. 281 × C. P. 28/44); 9 (Co. 281 × C. P. 28/36); and 24 (U. S. 1643 × C. P. 29/284) are actually giving a larger number of resistant seedlings.

TABLE 16.—*Number of field selections from 24 crosses of the 1929–34 Canal Point series falling in four classes of red rot resistance or susceptibility*

No. of cross	Cross and red rot rating of parents [1]	Class 1 (resistant)	Class 2 (moderately resistant)	Class 3 (susceptible)	Class 4 (very susceptible)	Total
1929 C. P. SERIES						
1	Co. 281 (1) ×P. O. J. 2878 (2)	7	8	1	10	26
2	Co. 281 (1)×U. S. 1694 (4)	4	10	10	2	26
3	P. O. J. 2725 (4)×C. P. 1165 (3)	1	11	33	3	48
4	P. O. J. 2725 (4)×C. P. 27/73 (?)	6	28	12	11	57
5	P. O. J. 2753 (4)×C. P. 830 (3)	0	2	9	40	51
Total		18	59	65	66	208
Percent		8.7	28.4	31.2	31.7	...
1931 C. P. SERIES						
6	Co. 281 (1)×P. O. J. 2878 (2)	15	30	33	17	95
7	Co. 281 (1)×U. S. 1694 (4)	0	12	45	17	74
Total		15	42	78	34	169
Percent		8.9	24.9	46.2	20.1	
1932 C. P. SERIES						
8	Co. 281 (1)×C. P. 28/44 (4)	12	12	10	9	43
9	Co. 281 (1)×C. P. 28/36 (1)	13	13	6	19	51
10	P. O. J. 2725 (4)×C. P. 1161 (1)	7	8	13	16	44
Total		32	33	29	44	138
Percent		23.2	23.9	21.0	31.9	
1933 C.P. SERIES						
11	C. P. 1165 (3)×C. P. 28/44 (4)	4	10	17	10	41
12	C. P. 1165 (3)×C. P. 27/108 (4)	6	5	17	12	40
13	C. P. 28/9 (3)×C. P. 31/288 (4)	3	10	8	15	36
14	C. P. 29/142 (2)×C. P. 27/108 (4)	5	12	12	21	50
15	C. P. 29/307 (3)×C. P. 27/48 (?)	4	4	7	22	37
16	C. P. 31/117 (4)×C. P. 28/100 (3)	8	4	3	12	27
17	C. P. 31/289 (2) selfed [2]	7	8	10	2	27
18	Co. 281 (1)×U. S. 1694 (4)	29	43	42	20	134
Total		66	96	116	114	392
Percent		16.8	24.5	29.6	29.1	
1934 C. P. SERIES						
19	C. P. 28/11 (1)×C. P. 27/38 (4)	11	7	5	4	27
20	Co. 281 (1)×C. P. 1165 (3)	11	4	10	12	37
21	Co. 281 (1)×C. P. 29/138 (2)	5	4	11	11	31
22	Co. 281 (1)×P. O. J. 2878 (2)	1	4	8	13	26
23	Co. 281 (1)×U. S. 1694 (4)	12	24	28	35	99
24	U. S. 1643 (4)×C. P. 29/284 (1)	18	13	25	55	111
Total		58	56	87	130	331
Percent		17.5	16.9	26.3	39.3	..
Grand total		189	286	375	388	1,238
Percent		15.3	23.1	30.3	31.3	

[1] The numbers in parentheses indicate the red rot ratings of the parent varieties.
[2] C. P. 31/289 is a seedling of Co. 281 × U. S. 1694.

Fairly large numbers of seedlings have been available for two of the crosses listed in this table, Co. 281×P. O. J. 2878 (Nos. 1, 6, and 22), and Co. 281×U. S. 1694 (2, 7, 18, and 23). From the former, a total of 147 selections was made during 3 years, of which 65, or 44 percent, were resistant or moderately resistant to red rot. This is approximately the same as the 46.5 percent found for the complete progeny of the 1931 series, discussed under the complete progeny studies (p. 79).

From the Co. 281×U. S. 1694 cross, 134 or 40.2 percent of the 333 selections made during 4 years (cross numbers 2, 7, 18, and 23) were in the resistant classes. This is somewhat higher than the 30.6 percent found for the entire progeny of the 1931 series, and nearly three times the 14.7 percent found for the selections from the 1931 series complete progeny.

Both of these crosses have given consistently a relatively high number of agronomic selections, the cross with U. S. 1694 being particularly valuable from the standpoint of the early maturity of many of the seedlings. In view of this fact, it is fortunate that they may also be depended upon to give a considerable number of seedlings combining desirable agronomic qualities with satisfactory red rot resistance. Presumably, the resistance of the seedlings from these crosses may be attributed largely to the resistant female parent, Co. 281.

The value of Co. 281 as a parent in transmitting resistance to its seedlings is further apparent from the figures for crosses 8 and 9, in which two of its seedlings, C. P. 28/36 and 28/44, were backcrossed on it. More than half of the selections from these crosses fell in the resistant classes. Co. 281, in fact, has been either a parent or grandparent in nearly every cross from which any considerable number of red rot resistant seedlings has been obtained.

REACTION OF AGRONOMIC SELECTIONS

The red rot ratings of 79 agronomic selections from the 1929 to 1934 Canal Point series, inclusive, are given in the following list: For the 1929, 1931, and 1932 series the laboratory ratings have been verified by field observations, but for the 1933 and 1934 series less opportunity has been afforded for comparing the field and laboratory reactions. In most cases, therefore, the ratings must be considered tentative.

In the entire group there are but two very susceptible (class 4 or 5) varieties. These two, C. P. 29/99 and 33/243, are canes of commercial promise except for their high susceptibility to red rot. The 29/99 has been found to possess excellent windrowing qualities (*45*), and it is more resistant to mosaic than Co. 281, the only variety suitable for windrowing now available for commercial use in Louisiana. Its high susceptibility to red rot, however, has made it dangerous to recommend to the growers.

Red rot-resistance trials with C. P. 33/243 in the field have indicated that germination of this early-maturing variety may be reduced 20 percent or more by inoculation either in the stalk or by smearing a culture of the red rot fungus over the root rings. While apparently not so susceptible as P. O. J. 213 to infection through the root buds, the fact that invasion of the stalks occurs readily from this source indicates that germination of the variety might be materially reduced during a cold wet winter. Further field trials with this apparently

valuable variety are being continued to determine whether its susceptibility to red rot should prevent its recommendation to growers.

The percentage of resistant canes (classes 1 and 2) among the agronomic selections increased from 27 in the 1929 series to 65 in the 1933 series, and 73 in the 1934 series. Thus, it is apparent that in the later series larger numbers of seedlings are being produced that combine desirable agronomic qualities with red rot resistance.

Variety:	*Red rot rating*
C. P. 29/89	Susceptible.
C. P. 29/94	Moderately resistant.
C. P. 29/99	Very susceptible.
C. P. 29/103	Susceptible.
C. P. 29/108	Susceptible.
C. P. 29/120	Susceptible.
C. P. 29/131	Susceptible.
C. P. 29/137	Susceptible.
C. P. 29/142	Moderately resistant.
C. P. 29/283	Susceptible.
C. P. 29/301	Moderately resistant.
C. P. 31/89	Resistant.
C. P. 31/110	Moderately resistant.
C. P. 31/114	Susceptible.
C. P. 31/146	Susceptible.
C. P. 31/152	Susceptible.
C. P. 31/160	Susceptible.
C. P. 31/161	Susceptible.
C. P. 31/258	Susceptible.
C. P. 31/296	Susceptible.
C. P. 31/509	Resistant.
C. P. 31/516	Moderately resistant.
C. P. 31/551	Susceptible.
C. P. 32/2	Susceptible.
C. P. 32/118	Moderately resistant.
C. P. 32/120	Resistant.
C. P. 32/123	Resistant.
C. P. 32/124	Resistant.
C. P. 32/126	Resistant.
C. P. 32/138	Moderately resistant.
C. P. 32/146	Resistant.
C. P. 32/182	Moderately resistant.
C. P. 32/202	Susceptible.
C. P. 32/206	Moderately resistant.
C. P. 32/209	Moderately resistant.
C. P. 32/324	Moderately resistant.
C. P. 32/334	Moderately resistant.
C. P. 32/336	Moderately resistant.
C. P. 33/51	Moderately resistant.
C. P. 33/53	Resistant.
C. P. 33/99	Susceptible.
C. P. 33/121	Resistant.
C. P. 33/165	Moderately resistant.
C. P. 33/224	Moderately resistant.
C. P. 33/228	Resistant.
C. P. 33/229	Moderately resistant.
C. P. 33/243	Very susceptible.
C. P. 33/253	Moderately resistant.
C. P. 33/278	Susceptible.
C. P. 33/301	Moderately resistant.
C. P. 33/309	Susceptible.
C. P. 33/310	Susceptible.
C. P. 33/320	Susceptible.
C. P. 33/366	Susceptible.
C. P. 33/372	Moderately resistant.
C. P. 33/389	Susceptible.

Variety—Continued.	*Red rot rating*
C. P. 33/394	Moderately resistant.
C. P. 33/406	Moderately resistant.
C. P. 33/409	Resistant.
C. P. 33/415	Resistant.
C. P. 33/445	Resistant.
C. P. 33/450	Susceptible.
C. P. 33/451	Resistant.
C. P. 33/471	Resistant.
C. P. 33/472	Resistant.
C. P. 33/485	Susceptible.
C. P. 33/494	Moderately resistant.
C. P. 33/500	Moderately resistant.
C. P. 34/1	Resistant.
C. P. 34/16	Susceptible.
C. P. 34/21	Moderately resistant.
C. P. 34/25	Resistant.
C. P. 34/27	Susceptible.
C. P. 34/59	Moderately resistant.
C. P. 34/75	Resistant.
C. P. 34/77	Moderately resistant.
C. P. 34/79	Moderately resistant.
C. P. 34/80	Moderately resistant.
C. P. 34/81	Susceptible.

NATURE OF RESISTANCE

Within recent years important advances have been made in an understanding of the nature of resistance to certain diseases in a number of crop plants, but little attention has been given to the subject in the study of diseases of sugarcane. The economic importance of red rot on sugarcane in the United States and the relative rarity of resistance to it, particularly in varieties combining this character with other desirable commercial qualities, emphasizes the desirability of an understanding of the nature of resistance to the disease. Aside from its scientific interest, such information might lead to the discovery of a relationship between resistance to red rot and one or more other characters that would be of value in the breeding and selection work.

The protective mechanism of sugarcane varieties against red rot may be concerned with resistance of the host tissues to infection by the parasite and to its development within the tissues of the host after infection has occurred.

RESISTANCE TO INFECTION

In earlier papers (*10*, *11*) the author reported that certain varieties of sugarcane possessed what may be termed a functional resistance to red rot, manifested by their escape from or prevention of invasion of the stalk by *Colletotrichum falcatum*, through the root primordia because of their early and vigorous root development. This was particularly true of the variety C. P. 807, the internal stalk tissues of which are very susceptible to red rot, but which does not ordinarily become infected through the root primordia under conditions favorable for root formation. Whether this was due to a mechanical escape from infection as a result of the development of the roots, or to actual resistance of the tissues of the root primordia to invasion has not been determined. In the experiments mentioned, invasion of the stalk through the root primordia occurred if the young rootlets were

injured, or if the cuttings were subjected to conditions that induced dormancy and prevented root development over a prolonged period.

The experimental results, demonstrating that this type of resistance (or escape from infection) might be broken down under certain conditions, were confirmed by actual field experience with C. P. 807 in 1935 and 1936, when prolonged dormancy of the planted seed cuttings in heavy soil on several plantations was followed by red rot invasion of the stalks through the nodes. Such infection of seed cuttings of this variety has occurred extensively, however, in only 2 of the 6 years C. P. 807 has been cultivated on a commercial scale. While such functional resistance or escape from infection, therefore, is influenced by environmental conditions and cannot be depended on to protect the plant against the disease under all conditions, it may be of considerable importance in the protective mechanism of some varieties. Commercial experience with sugarcane varieties makes it evident, however, that resistance of the tissues to invasion as well as to development of the fungus after infection is of primary importance in combating red rot. Studies were undertaken with the view of determining the nature of this type of resistance to red rot in sugarcane varieties. The pathological anatomy was first considered.

RESISTANCE OF HOST TISSUES

Pathological Anatomy

Previous studies of the pathological anatomy of red rot have not been concerned with a comparison of the disease in susceptible and resistant hosts, and in very few instances has the variety of sugarcane studied been named. Went (*73*) in the original study of the disease found that the fungus developed principally in the parenchyma cells of the stalk, but noted that the vascular bundles might be invaded also. Within the invaded cells the mycelium was characterized by the presence of oil droplets, which are soluble in alcohol and ether. Frequently the hyphal walls dissolved, leaving the oil droplets in the cells in rows, marking the course of the former hyphae. This is in accord with the author's observations. Butler's account (*29*) of the pathological anatomy agrees essentially with that of Went.

For the present studies a very susceptible variety, P. O. J. 213, and a resistant one, Co. 281, were chosen. These varieties have certain similar morphological characters, such as stalk size, color, and fiber content; and while there was no evidence to indicate that any of these characters was correlated with red rot resistance, it seemed desirable to choose varieties that were similar in as many respects as possible, aside from their resistance to red rot. Free-hand sections of both naturally and artificially infected stalks were prepared.

As noted under the discussion of the life history, the red rot fungus invades the sugarcane stalk through wounds, or through the root primordia. Regardless of the method of entrance into the stalk tissues, it soon invades both the parenchyma cells and the vascular bundles. This occurs in growing cane as well as in cuttings, although, as pointed out by Edgerton and Moreland (*36*), spread through the parenchyma is more rapid in cuttings than in standing cane. In both standing cane and cuttings the fungus develops more rapidly in P. O. J. 213 than in Co. 281. The rate of spread appears to be governed by the characters of the cell contents rather than of their

walls, since no indication was found that the cell walls of Co. 281 offered any particular mechanical resistance to the progress of the fungus. Within the invaded cells of the two varieties, however, there is a marked difference in the rate and extent of the branching of the hyphae. In the susceptible P. O. J. 213 the hyphae develop rapidly, branching profusely and frequently producing an abundance of conidia. In Co. 281, on the other hand, the progress of the fungus is much slower, usually only short lateral branches are produced in the freshly invaded cells, and conidial production is scanty. Eventually the invaded cells of the resistant host may be filled with mycelium, as in the susceptible one, but the rate of development is much slower. These observations indicated that the resistance principle was contained within the protoplasm.

Phenolic Compounds in Relation to Resistance

A study of phenolic compounds in resistant and susceptible hosts suggested itself in view of the known occurrence of such compounds in the sugarcane plant, and the fact that certain polyphenols have been found to play an important role in the resistance of some plants to fungus diseases. Three preliminary experiments have been conducted.

In the first series of experiments, carried out in December 1936, a very susceptible variety, P. O. J. 213, and a resistant one, Co. 281, were used. The rind was peeled from internodes in the lower and upper portions of mature stalks, and the juice extracted by passing the stalk tissues through a food grinder. After straining through cheesecloth, total phenols were determined on duplicate portions of the juice from each variety according to the method of Newton and Anderson (*50*).

In the bottom internodes the phenol content in the resistant Co. 281 was approximately 50-percent greater than in the susceptible P. O. J. 213, and in the upper internodes 700-percent greater. Repetition of the experiment gave similar results.

In a second series of experiments conducted in September 1937, the red rot resistant C. P. 28 11 and the very susceptible C. P. 807 were used in addition to the Co. 281 and P. O. J. 213. Because of the immaturity of the cane at this season, only the lower internodes were extracted. The phenol content of the C. P. 28 11 was approximately 25-percent greater than that of the P. O. J. 213 and 12-percent greater than the C. P. 807, while that of the Co. 281 was 11-percent greater than the P. O. J. 213 and 6-percent greater than the C. P. 807.

These results must be considered entirely preliminary in character and no attempt is made to draw conclusions from them. While it appears significant that in three experiments red rot-resistant varieties showed a higher phenol content than the susceptible ones, larger numbers of varieties in both red rot classes must be studied before generalizations are permissible. Furthermore, as pointed out by Walker and Link (*72*), it is advisible to reserve judgment on the protective role of a phenolic substance in the defense mechanism of plants against diseases until the compound or compounds have been isolated from the host tissue in the pure state and their toxicity to the parasite in question determined. The results obtained thus far, however, are such as to warrant further investigation of this phase of the problem.

CONTROL OF THE DISEASE

SEED SELECTION

Since the time that red rot was first recognized as a disease of seed cane, the use of sound seed for planting has been emphasized as one of the principal means of control. This is important not only with respect to red rot infection but also as to borer damage.

The difficulty under most plantation conditions in Louisiana is to obtain borer-free and red rot-free seed, particularly in years of heavy borer infestation. A survey of possible sources of seed supply on the plantation, however, will usually show some fields to be less infested than others. Where this is true, the improved stands of cane that will be obtained from the use of the sounder seed will more than compensate for extra hauling and other incidental costs involved.

In the sirup sections, the question of seed selection is much simpler. The smaller scale on which cane is grown there permits the examination of each cutting as it is taken from the seedbeds in the spring. Great care must be exercised in the examination of the cuttings, discarding those that show indications of red rot infection. Of the present varieties grown to any extent for sirup production, Cayana and Louisiana Purple (or Georgia Red) suffer the most severe seed deterioration in the beds and subsequent losses in stands. P. O. J. 213, as mentioned previously, escaped such injury until the spring of 1938, when considerable deterioration in the seedbeds and later reductions in stands occurred. Spoilage of Co. 290 seed also occurs commonly in the beds, but this is believed to be due principally to causes other than red rot. Thus far the superior-yielding, high-quality sirup variety C. P. 29/116 has shown no serious deterioration from red rot or other diseases in the sirup-producing areas, and the replacement of Cayana and Louisiana Purple with it is to be recommended. In spite of the seed-cane spoilage that may occur with P. O. J. 213 and Co. 290, however, these varieties, because of their superiority in yields, may also profitably replace Cayana and Louisiana Purple.

SEED PLOTS

Barber (*22*) in India was perhaps the first to point out the value of setting aside certain fields of cane to be used solely for seed purposes as a means of controlling red rot. This was later advocated by Edgerton (*33*) and Edgerton, Taggart, and Tims (*37*) as a general disease-control measure in Louisiana. In recent years a very important step toward obtaining better seed cane supplies has been taken in Louisiana by several growers in the establishment of seed plots, principally in isolated areas as a means of keeping seed supplies free from mosaic. While limited at present largely to mosaic susceptible varieties, it is probable that the future will see the practice expanded to include other varieties. In addition to the benefits that will accrue from the control or reduction of mosaic, the growing of cane solely for seed purposes will permit special attention to be given it with respect to fertilization and cultivation, and should result in generally higher quality of seed cane with consequent improvement in germination and stands.

CULTURAL PRACTICES

A great deal can be done toward reducing red rot injury to seed cane by attention to cultural practices that tend to stimulate germination (*11*, *13*). Among these "good farming" practices may be mentioned thorough preparation of the seedbed well in advance of planting time so that it will be well settled, and avoiding too deep covering of the seed at planting time. Every year gappy stands result on some plantations owing to too deep covering. This results in retardation of germination, thus favoring fungus deterioration of the seed.

SUMMER PLANTING

Planting a part of the acreage in August, when excellent germination can be obtained from immature seed cane, offers a means of escaping considerable injury from seed-rotting diseases which affect cane planted in the fall. This is particularly true of varieties susceptible to both red rot and root rot (*55*). Summer planting enables the plants to become well established on their own root systems before the winter months, and once this is accomplished deterioration of the seed cuttings is of little consequence. Not all varieties respond well to summer planting, however, and they should be chosen for their adaptability in this respect (*16*).

DESTRUCTION OF TRASH

The destruction of cane trash and diseased material after harvest has been recommended (*22*, *26*, *30*, *33*, *58*, *61*) as a means of reducing red rot infection. For several reasons, it is doubtful whether trash destruction for the sole purpose of reducing red rot would have enough effect on the disease, under Louisiana plantation conditions at least, to warrant any special effort being made in this regard. In the first place, it is practically impossible to do a complete job of disposing of cane refuse, and the small quantity that would always remain would probably carry enough of the red rot fungus to start leaf infections in the spring. Furthermore, seed cuttings and stubble rhizomes that become exposed during spring cultivation operations serve as sources of inoculum for leaf infections, and when the disease is once started on the leaves it soon spreads rapidly through the fields. Many arguments have been advanced for and against the burning of trash for reasons other than red rot control, and so far as the sugar district of Louisiana is concerned, the question should be decided on its merits aside from its relation to this disease. In the sirup sections, burning of trash and diseased cuttings discarded from the seedbeds is to be recommended.

RESISTANT VARIETIES

In recent years the greatest dependence for the control of red rot has rested on the utilization of resistant varieties. Unless a variety possesses a satisfactory degree of resistance to red rot, other control measures will be ineffective in many years. This is particularly true on the heavy, poorly drained lands in the Louisiana sugar district, where susceptibility to both red rot and root rot must be considered in the choice of varieties. Past experience has shown that large-scale planting of canes that are susceptible to these diseases may prove extremely costly.

The present assortment of varieties suitable for commercial culture in the United States still leaves much to be desired with respect to resistance to red rot and other diseases. During the past decade, however, such great progress has been made that the industry in the United States is now on a firmer basis from this standpoint than at any time in its history. Judging from the number of disease-resistant, commercially promising seedlings now being tested, with the prospect that others will be produced each year, the future outlook for the effective reduction in losses from red rot and other diseases appears very promising.

SUMMARY

Red rot, caused by *Colletotrichum falcatum*, is one of the major diseases of sugarcane in the southern United States, where, since the time of its discovery in the country early in the present century, it has been an annual source of losses to the industry and at times an important contributing factor to the decline of several sugarcane varieties. The symptoms of the disease on the leaves and in the stalks are described, and the losses caused by it in reduced stands and yields of cane and sugar are discussed.

In 1929–31 unusually severe losses were sustained from it in Louisiana through the sudden failure of one of the leading commercial varieties, P. O. J. 213. In seeking the cause of this failure, a detailed survey of the red rot flora of the southern United States was undertaken, in the course of which isolates of *Colletotrichum falcatum* were obtained from the leading commercial varieties in Louisiana, the sirup-producing States, and southern Florida. This revealed the presence of two morphological races of the fungus, distinguished by the color and texture of the mycelium in artificial culture. On the basis of color, these were termed light and dark races, respectively. Isolates intermediate in character between the two races were also obtained.

During the early years of the survey (1930–34) the light race predominated in Louisiana, but beginning in 1934 the dark race increased in relative numbers, and is now more prevalent than the light. Evidence is presented to indicate that this change was influenced by the changes in relative acreages of commercial varieties of sugarcane that occurred from 1933–36. In spite of this change in the red rot flora as a whole, P. O. J. 213 continued to show a preference for the light-race isolates even when abundantly exposed to the dark ones; and conversely the dark-race isolates predominated on C. P. 807 under conditions of exposure to both. It was concluded from the survey that the light race was responsible for the failure of P. O. J. 213, and the dark race for the decline of C. P. 807.

In the sirup-producing States the dark race predominated from 1930–37, but in 1938 the light race predominated on all varieties except Cayana. In southern Florida, only light-race isolates were obtained.

Detailed cultural studies of *C. falcatum* are reported. Measurements of the conidia of 81 isolates showed no correlation between conidial size and the cultural characters differentiating the two races, nor could they be differentiated on the basis of the rate of growth in culture at different temperatures. The optimum temperature for the fungus was from 30° to 32.5° C.; 37° was unfavorable to growth, growth was slow at 15°; and no growth was obtained below 10° C.

The cultural characters of single-spore cultures of the fungus remained constant throughout the period of the investigation, mutation or other forms of marked variation not being observed.

Both dark- and light-race isolates of *Colletotrichum falcatum* were obtained from lesions on the leaf midribs of *Sorghum halepense*, *S. vulgare*, and *Erianthus giganteus* in Louisiana that were indistinguishable in morphology and parasitism from those obtained from leaves and stalks of sugarcane. The grass and sugarcane isolates were compared in culture with a culture of *C. graminicolum* obtained from the Netherlands and one from Iowa. Morphologically the former culture was similar to, although not identical with, some of the dark-race isolates of *C. falcatum*, whereas the Iowa culture showed little resemblance to *C. falcatum*. In inoculation experiments neither culture of *C. graminicolum* developed in the tissues of red rot-susceptible varieties of sugarcane. It was concluded that the two species are distinct, and that the fungus commonly occurring on the leaf midribs of Johnson grass, plumegrass, and sorgo in southern Louisiana is *C. falcatum*.

In life-history studies, it was found that the disease on the leaves of sugarcane was of importance principally as a means of dissemination of conidia, providing an abundant source of inoculum for stalk infections. Infection of the leaves commonly occurs through the wounds made by insects, but penetration through the apparently uninjured epidermis may occur. Stalk infections of standing cane occur largely through the tunnels of the moth borer (*Diatraea saccharalis* (F.)), and in certain very susceptible varieties, through the root primordia. In seed cuttings, it may also occur through the cut ends. The disease develops during the winter in seed cane from infections that have occurred prior to cutting and planting. In the spring, conidia produced on trash and pieces of seed cuttings exposed in cultivation operations, and on stubble rhizomes serve as sources of inoculum for initiating infection of the leaves on which the disease continues to multiply throughout the growing season. No evidence was obtained to indicate that the fungus is able to persist in the soil. Rotting of seed cuttings under field conditions is favored by poor drainage.

The comparative virulence of both the light and the dark cultural races of the fungus was determined on a resistant host, Co. 281, and a very susceptible one, P. O. J. 213. On these hosts, the isolates of the light race from Louisiana were more virulent, in general, than those of the dark, and the latter were more virulent than either the light- or dark-race isolates from the sirup-producing States. The representatives of the two cultural races tested from the sirup-producing States were of about equal virulence. From these results it was apparent that there was, in general, a relation between virulence and the geographic origin of the isolates with respect to the principal sugarcane-growing areas of the United States.

Although well-defined physiologic forms, such as have been described for the rust fungi, could not be differentiated, a degree of parasitic specialization within each cultural race was demonstrated. When a group of light-race isolates obtained from fields of P. O. J. 213 in Louisiana where it failed, for example, were compared with those obtained from other commercial varieties, the P. O. J. 213 isolates as a group were significantly more virulent than groups from Co. 281, D-74, and miscellaneous varieties, with the exception of those

from P. O. J. 36–M. This indicated that a measureable degree of parasitic specialization of the P. O. J. 213 light-race forms had occurred on that variety and together with the results of the survey showing that the light cultural race was dominant on P. O. J. 213 during the period of its failure, offered a plausible explanation for its decline.

The dark-race isolates obtained from C. P. 807, furthermore, from sections where red rot was severe on this variety, were significantly more virulent on it than those from areas where the disease was less severe. While there was this evidence of a degree of parasitic specialization among certain of the isolates from C. P. 807, a comparison of all of the isolates from this variety with those from other varieties indicated that as a group the C. P. 807 isolates did not constitute a specialized parasitic race.

Additional evidence of specialization was found in the isolation of one Louisiana light-race culture from Co. 281 which proved to be highly virulent toward that generally resistant variety. The evidence indicated, however, that this was a chance variant and not a representative of a large population in nature.

On P. O. J. 213 the dark-race isolates from Louisiana were significantly more virulent than either the light- or dark-race isolates from the sirup-producing States. This suggested that a partial explanation for the absence for many years of red rot injury to the P. O. J. 213 in the sirup-producing States lay in the lower virulence toward it of the races of *C. falcatum* present there. This tentative conclusion was corroborated by a test in which certain Louisiana light-race isolates produced infection of this variety through the root primordia and subsequent rotting of the stalk to a greater degree than representatives of both races from Georgia.

Among the dark-race isolates, differences in relative virulence were found similar to those described for the isolates of the light cultural race. As a group, the isolates from Louisiana were more virulent than those from the sirup-producing States; and among certain of the Louisiana dark forms there was further evidence of a relation between virulence and geographic origin.

The importance of red rot in the Department's sugarcane-breeding and disease-testing program is discussed, and the laboratory method of testing large numbers of seedlings for red rot resistance is described. Based on the results of these tests the seedlings are classified into 5 groups of resistance or susceptibility. The laboratory tests are supplemented by field-inoculation tests with the most promising agronomic selections, to determine the effect of the disease under actual field conditions where the extent of injury is influenced by such factors as the vigor of the sugarcane variety, susceptibility to borer attack, susceptibility to root rot, and soil and weather conditions. It was found that susceptible varieties that are very vigorous may suffer less from red rot than more resistant ones that are less vigorous. Varieties that are moderately resistant to red rot but susceptible to root rot may be more severely injured by red rot than those susceptible to the disease but resistant to root rot.

The red rot reaction of varieties representing the five generally accepted species of *Saccharum* was determined by the laboratory method. Thirty-two varieties of *S. officinarum* studied were classed as very susceptible and 4 as susceptible, from which it was concluded

that high susceptibility to the disease is characteristic of this species. Twenty-eight New Guinea varieties, tentatively classed as noble canes, were very susceptible, 3 were susceptible, 1 moderately resistant, and 1 resistant.

Of the two varieties of *S. barberi* studied, one was very susceptible and one moderately resistant. Two varieties of *S. sinense* were very susceptible and seven were susceptible, indicating that the members of this species are only slightly less susceptible than those of *S. officinarum*. The one variety of *S. robustum* available for testing was classed as susceptible.

With one exception, the varieties of *S. spontaneum* were found to be more resistant then those of the other species, 13 of the 14 tested being classed as moderately resistant, and 1 as very susceptible. A study of the genealogy of the resistant seedlings produced at the Department's breeding station indicated that some form of *S. spontaneum* was the most probable source of their resistance to the disease.

The distribution of red rot resistance among the seedlings of four complete progenies was studied, two of them, Co. 281 × P. O. J. 2878 and Co. 281 × U. S. 1694, in considerable detail. No correlation was found to exist between red rot resistance and the growth habit of the female parent (Co. 281), the agronomic character of the seedlings, or the sucrose content of their juices.

From a study of the distribution of resistance among the field selections of 24 commercial crosses, it was concluded that Co. 281 is of great value as a parent in breeding for red rot resistance. This variety entered into the parentage of nearly all of the crosses from which any considerable number of red rot resistant seedlings was obtained.

The red rot reaction is given of 79 of the most promising agronomic selections from the 1929–34 Canal Point series. The percentage of resistant canes among these selections increased from 27 in the 1929 series to 73 in the 1934 series, indicating that in the later series larger numbers of seedlings were produced, which combined desirable agronomic qualities with red rot resistance.

Studies of the nature of resistance to red rot among sugarcane varieties indicated that it might be concerned with resistance of the host to invasion of the parasite through the root primordia, and to the development of the fungus within the host tissues after invasion had occurred. The first type of resistance is illustrated by C. P. 807, which is resistant to invasion through the root primordia under average field conditions, but which may be invaded under environmental conditions unfavorable to the host and favorable to the parasite, such as prolonged low temperatures accompanied by excessive soil moisture. Resistance of the host tissues to the spread of the parasite after invasion, therefore, was considered of greater importance.

A comparative study of the pathological anatomy of a resistant and a very susceptible host did not reveal differences in the character of the tissues of the two varieties that would explain their different reaction to the diseases. It was concluded that the resistance principle was contained within the protoplasm.

Preliminary determination of phenolic compounds in the extracted juices of two resistant and two very susceptible varieties are reported, in which the former were found to have a higher total phenolic content than the latter. While the results suggested that such compounds

might be concerned in the resistance mechanism, the preliminary nature of the experiments did not permit such a conclusion to be drawn.

In considering measures for the control of red rot, seed selection, the establishment of plots where cane is grown especially for seed, sound cultural practices, and summer planting of susceptible varieties are discussed. It is recognized, however, that the control of the disease must depend principally on the utilization of resistant varieties, the production of which is one of the cardinal aims of the Department's breeding program.

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